Monobind Inc.
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AccuBind
ELISA Microwells

Cancer Antigen 15-3 (CA 15-3) Test System
Product Code: 5625-300

1.0 INTRODUCTION

Intended Use: The Quantitative Determination of Cancer Antigen (CA 15-3) Concentration in Human Serum by a Microplate Enzyme Immunoassay, Colorimetric

2.0 SUMMARY AND EXPLANATION OF THE TEST

Although multiple serum based tumor markers have been described for breast cancer, such as CA 15-3, BR 27-29, carcinoembryonic antigen (CEA), tissue polypeptide antigen (TPA), tissue polypeptide specific antigen, and HER-2 (the extracellular domain), the most widely used are CA 15-3 and CEA. CA 15-3 is considered to be one of the first circulating prognostic factors for breast cancer.[1] Preoperative concentrations thus might be combined with prognostic factors for predicting outcome in patients with newly diagnosed breast cancer.[2] At present the most important clinical application of CA 15-3 is in monitoring therapy in patients with advanced breast cancer that is not accessible by existing clinical or radiologic procedures.[3]

The CA 15-3 assay measures the protein product of *MUC1* gene. *MUC1* protein is a large transmembrane glycosylated molecule containing three main domains, a large extracellular region, a membrane spanning sequence, and a cytoplasmic domain.[4] Although the physiologic function of *MUC1* is unclear, the glycoprotein has been implicated in cell adhesion, immunity and metastasis. Compared with healthy breast tissue, *MUC1* is present in higher concentrations but less glycosylated in breast carcinoma.[5-8]

In this method, a prediluted CA15-3 calibrator diluted patient specimen or control is first added to a streptavidin coated well. Biotinylated monoclonal antibody (specific for CA15-3) is added and the reactants mixed. Reaction between the CA15-3 antibodies and native CA15-3 forms complex that binds with the streptavidin coated to the well. The excess serum proteins are washed away via a wash step. Another enzyme labeled antibody specific for a different epitopic recognition of CA15-3 is added to the wells. The enzyme labeled antibody binds to the CA15-3 already immobilized on the well through its binding with the biotinylated monoclonal antibody. Excess enzyme is washed off via a wash step. A color is generated by the addition of a substrate. The intensity of the color generation is directly proportional to the concentration of the CA15-3 in the sample.

3.0 PRINCIPLE

Immunoenzymometric sequential assay (TYPE 4):
The essential reagents required for an immunoenzymometric assay include high affinity and specificity antibodies (enzyme and immobilized), with different and distinct epitope recognition, **in excess,** and native antigen. In this procedure, the immobilization takes place during the assay at the surface of a microplate well through the interaction of streptavidin coated on the well and exogenously added biotinylated monoclonal anti-CA15-3 antibody.

Upon mixing monoclonal biotinylated antibody, and a serum containing the native antigen, a reaction results between the native antigen and the antibody, forming an antibody-antigen complex. The interaction is illustrated by the following equation:

$$Ag_{(CA15\text{-}3)} + {}^{Btn}Ab_{(m)} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} Ag_{(CA15\text{-}3)} - {}^{Btn}Ab_{(m)}$$

$^{Btn}Ab_{(m)}$ = Biotinylated Monoclonal Antibody (Excess Quantity)
$Ag_{(CA15\text{-}3)}$ = Native Antigen (Variable Quantity)
$Ag_{(CA15\text{-}3)} - {}^{Btn}Ab_{(m)}$ = Antigen-antibody complex (Variable Quantity)
k_a = Rate Constant of Association
k_{-a} = Rate Constant of Disassociation

Simultaneously, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antibody. This interaction is illustrated below:
$Ag_{(CA15\text{-}3)} - {}^{Btn}Ab_{(m)} + Streptavidin_{CW} \Rightarrow$ Immobilized complex (IC)
$Streptavidin_{CW}$ = Streptavidin immobilized on well
Immobilized complex (IC) = Ag-Ab bound to the well

After a suitable incubation period, the antibody-antigen bound fraction is separated from unbound antigen by decantation or aspiration. Another antibody (directed at a different epitope) labeled with an enzyme is added. Another interaction occurs to form an enzyme labeled antibody-antigen-biotinylated-antibody complex on the surface of the wells. Excess enzyme is washed off via a wash step. A suitable substrate is added to produce color measurable with the use of a microplate spectrophotometer. The enzyme activity on the well is directly proportional to the native free antigen concentration. By utilizing several different serum references of known antigen concentration, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

$$(IC) + {}^{Enz}Ab_{(x\text{-}CA15\text{-}3)} \underset{k_b}{\overset{k_b}{\rightleftharpoons}} {}^{Enz}Ab_{(x\text{-}CA15\text{-}3)} - IC$$

$^{Enz}Ab_{(x\text{-}CA15\text{-}3)}$ = Enzyme labeled Antibody (Excess Quantity)
$^{Enz}Ab_{(x\text{-}CA15\text{-}3)}$ - IC = Antigen-Antibodies Complex
k_b = Rate Constant of Association
k_{-b} = Rate Constant of Dissociation

4.0 REAGENTS

Materials Provided:

A. CA 15-3 Calibrators – 1.0 ml/vial - Icons A-F
Six (6) vials of human serum based reference calibrators at concentrations of 0 (**A**), 10 (**B**), 40 (**C**), 100 (**D**), 200 (**E**) and 400 (**F**) U/ml. Store at 2-8°C. A preservative has been added.
Note 1: The calibrators are provided prediluted.
Note 2: The calibrators, human serum based, were made using a purified preparation of CA 15-3. The preparation was calibrated against Centocor CA 15-3 IRMA test.

B. CA 15-3 Biotin Reagent – 12 ml/vial – Icon ▽
One (1) vial contains biotinylated anti-human CA15-3 mIgG in a protein-stabilized matrix. A preservative has been added. Store at 2-8°C.

C. CA15-3 Enzyme Reagent – 12 ml/vial - Icon Ⓔ
One (1) vial contains horseradish peroxidase incorporated anti-human CA15-3 mIgG in a protein-stabilized matrix. A preservative has been added. Store at 2-8°C.

D. Streptavidin Coated Plate – 96 wells – Icon ⇓
One 96-well microplate coated with 1 µg/ml streptavidin and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

E. Wash Solution Concentrate – 20ml - Icon
One (1) vial contains surfactant in buffered saline. A preservative has been added. Store at 2-8°C.

F. CA 15-3 Dilution Matrix – 50 ml
One (1) vial of serum diluent contains buffer salts, protein, surfactants. Store at 2-8°C.

G. Substrate Solution – 12ml/vial - Icon S^N
One (1) vial contains tetramethylbenzidine (TMB) and hydrogen peroxide (H_2O_2) in buffer. Store at 2-8°C.

H. Stop Solution – 8ml/vial - Icon
One (1) vial contains a strong acid solution. Store at 2-8°C.

I. Product Instructions

Note 1: Do not use reagents beyond the kit expiration date.

Note 2: Avoid extended exposure to heat and light. Opened reagents are stable for sixty (60) days when stored at 2-8°C. **Kit and component stability are identified on the label.**
Note 3: Above reagents are for a single 96-well microplate.

4.1 Required But Not Provided:
1. Pipette capable of delivering 0.050ml (25µl) and 0.050ml (50µl) with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100ml (100µl) and 0.350ml (350µl) volumes with a precision of better than 1.5%.
3. Pipette (1000µl) used for serum diluent in patient dilutions.
4. Microplate washer or a squeeze bottle (optional).
5. Microplate Reader with 450nm and 620nm wavelength absorbance capability.
6. Absorbent Paper for blotting the microplate wells.
7. Plastic wrap or microplate cover for incubation steps.
8. Vacuum aspirator (optional) for wash steps.
9. Timer.
10. Quality control materials.

5.0 PRECAUTIONS

For In Vitro Diagnostic Use
Not for Internal or External Use in Humans or Animals

All products that contain human serum have been found to be non-reactive for Hepatitis B Surface Antigen, HIV 1&2 and HCV Antibodies by FDA required tests. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Edition, 1988, HHS Publication No. (CDC) 88-8395.

Safe disposal of kit componenets must be according to local regulatory and statutory requirement.

6.0 SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood serum or heparanised plasma in type and taken with the usual precautions in the collection of venipuncture samples. For accurate comparison to establish normal values, a fasting morning serum sample should be obtained. The blood should be collected in a redtop (with or without gel additives) venipuncture tube or for plasma use evacuated tube(s) containing heparin. Allow the blood to clot for serum samples. Centrifuge the specimen to separate the serum or plasma from the cells.

In patients receiving therapy with high biotin doses (i.e. >5mg/day), no sample should be taken until at least 8 hours after the last biotin administration, preferably overnight to ensure fasting sample.

Samples may be refrigerated at 2-8°C for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20°C for up to 30 days. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.050ml (50µl) of the diluted specimen is required.

7.0 QUALITY CONTROL

Each laboratory should assay controls at levels in the low, normal and elevated range for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

8.0 REAGENT PREPARATION

1. Wash Buffer
Dilute contents of wash solution to 1000ml with distilled or deionized water in a suitable storage container. Diluted buffer can be stored at room temperature (2-30°C) for up to 60 days.

2. Patient Sample Dilution (1:21)
Dispense 0.025ml (25µl) of each control and/or patient specimen into 0.50ml (500µl) of CA 15-3 dilution matrix appropriately labeled, clean container(s) and mix thoroughly before use. Store refrigerated at 2-8°C for up to 48 hours.

9.0 TEST PROCEDURE

Before proceeding with the assay, bring all reagents, serum reference calibrators and controls to room temperature (20-27°C).
*****Test Procedure should be performed by a skilled individual or trained professional.*****

1. Format the microplates' wells for each serum reference calibrator, control and patient specimen to be assayed in duplicate. **Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.**
2. Pipette 0.025 ml (25 µl) of the appropriate diluted calibrator, control or specimen into the assigned well.
3. Add 0.100 ml (100µl) of the biotinylated labeled antibody to each well. **It is very important to dispense all reagents close to the bottom of the coated well.**
4. Swirl the microplate gently for 20-30 seconds to mix and cover.
5. Incubate 60 minutes at room temperature.
6. Discard the contents of the microplate by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper.
7. Add 0.350ml (350µl) of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.**
8. Add 0.100 ml (100µl) of the Ca15-3 Enzyme Reagent to each well.
   DO NOT SHAKE THE PLATE AFTER ENZYME ADDITION
9. Cover and incubate 60 minutes at room temperature.
10. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.
11. Add 350µl of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.**
12. Add 0.100 ml (100µl) of substrate reagent to all wells. **Always add reagents in the same order to minimize reaction time.**
    DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION
13. Incubate at room temperature for twenty (20) minutes.
14. Add 0.050ml (50µl) of stop solution to each well and gently mix for 15-20 seconds.
15. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) in a microplate reader. **The results should be read within thirty (30) minutes of adding the stop solution.**

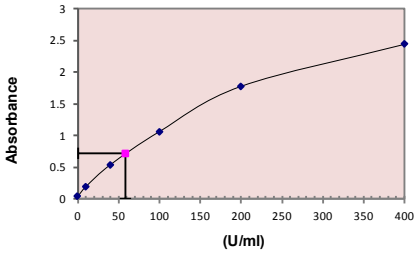
10.0 CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of CA15-3 in unknown specimens.
1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the absorbance for each duplicate serum reference versus the corresponding CA 15-3 concentration in U/ml on linear graph paper (do not average the duplicates of the serum references before plotting).
3. Connect the points with a best-fit curve.
4. To determine the concentration of CA 15-3 for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in U/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance (0.721) intersects the dose response curve at (58.4U/ml) CA 15-3 concentration (See Figure 1).

EXAMPLE 1

Sample I.D.	Well Number	Abs (A)	Mean Abs (B)	Value (U/ml)
Cal A	A1	0.044	0.043	0
	B1	0.042		
Cal B	C1	0.204	0.198	10
	D1	0.191		
Cal C	E1	0.560	0.543	40
	F1	0.525		
Cal D	G1	1.103	1.064	100
	H1	1024		
Cal E	A2	1.784	1.777	200
	B2	1.770		
Cal F	C2	2.431	2.438	400
	D2	2.445		
Patient	A3	0.737	0.721	**58.4**
	B3	0.705		

Figure 1



*The data presented in Example 1 and Figure 1 are for illustration only and **should not** be used in lieu of a dose response curve prepared with each assay.

11.0 Q.C. PARAMETERS

In order for the assay results to be considered valid the following criteria should be met:

1. The absorbance (OD) of calibrator F should be ≥ 1.3.
2. Four out of six quality control pools should be within the established ranges.

12.0 RISK ANALYSIS

The MSDS and Risk Analysis Form for this product are available on request from Monobind Inc.

12.1 Assay Performance

1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. The addition of substrate solution initiates a kinetic reaction, terminated by the addition of the stop solution. Therefore, the substrate and stop solution should be added in the same sequence to eliminate any time-deviation during reaction.
6. Plate readers measure vertically. Do not touch the bottom of the wells.
7. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
8. Use components from the same lot. No intermixing of reagents from different batches.
9. Patient specimens (diluted) with CA 15-3 concentrations above 400 U/ml may be further diluted (1/10 or higher) with CA15-3 diluted serum diluent and re-assayed. The sample's concentration is obtained by multiplying the result by the dilution factor.
10. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from Monobind IFU may yield inaccurate results.
11. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.
12. It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.
13. Risk Analysis- as required by CE Mark IVD Directive 98/79/EC - for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com.

12.2 Interpretation

1. ***Measurement and interpretation of results must be performed by a skilled individual or trained professional.***
2. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.
3. The reagents for the test system have been formulated to eliminate maximal interference; however, potential interaction between rare serum specimens and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for all kinds of immunoassays (Boscato LM, Stuart MC. 'Heterophilic antibodies: a problem for all immunoassays' Clin. Chem. 1988:3427-33). For diagnostic purposes, the results from this assay should be in combination with clinical examination, patient history and all other clinical findings.
4. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
5. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, Monobind shall have no liability.
6. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.
7. CA 15-3 has a low clinical sensitivity and specificity as a tumor marker. Clinically an elevated **CA 15-3 value alone is not of diagnostic value as a test for cancer and should only be used in conjunction with other clinical manifestations (observations) and diagnostic parameters.**

13.0 EXPECTED RANGES OF VALUES

The serum CA 15-3 is elevated in 2% of normal healthy women and 7% of patients with non-neoplastic conditions. Also, it has been reported to be elevated in cases of liver, lung, ovarian and colorectal cancers. No definitive ranges have been reported for those conditions.

TABLE I
Expected Values for the CA 15-3 Elisa Test System

Healthy Females	≤ 37 U/ml

It is important to keep in mind that establishment of a range of values which can be expected to be found by a given method for a population of "normal"-persons is dependent upon a multiplicity of factors: the specificity of the method, the population tested and the precision of the method in the hands of the analyst. For these reasons each laboratory should depend upon the range of expected values established by the Manufacturer only until an in-house range can be determined by the analysts using the method with a population indigenous to the area in which the laboratory is located.

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

The within and between assay precision of the CA 15-3 AccuBind® ELISA test system were determined by analyses on three different levels of control sera. The number, mean value, standard deviation (σ) and coefficient of variation for each of these control sera are presented in Table 2 and Table 3.

TABLE 2
Within Assay Precision (Values in U/ml)

Sample	N	X	σ	C.V.
Level 1	20	20.9	1.91	9.1%
Level 2	20	61.7	2.03	3.3%
Level 3	20	96.9	2.67	2.8%

TABLE 3
Between Assay Precision* (Values in U/ml)

Sample	N	X	σ	C.V.
Level 1	10	22.2	2.0	9.1%
Level 2	10	58.5	3.85	6.6%
Level 3	10	104.6	9.33	8.9%

*As measured in ten experiments in duplicate.

14.2 Sensitivity

The CA 15-3 procedure has a analytical sensitivity of 0.2 U/ml at three (3) SD from the zero calibrator. The functional sensitivity (20% CV) was found to be 1.25U/ml.

14.3 Accuracy

The CA 15-3 AccuBind® ELISA test system was compared with a reference method. Biological specimens from low, normal, and elevated concentrations were assayed. The total number of such specimens was 43. The least square regression equation and the correlation coefficient were computed for the CA 15-3 in comparison with the reference method. The data obtained is displayed in Table 4.

TABLE 4

Method	Mean	Least Square Regression Analysis	Correlation Coefficient
Monobind (y)	180.2	y=-0.219+1.008(x)	0.99
Reference (x)	178.6		

14.4 Specificity

In order to test the specificity of the antibody pair used massive concentrations of possible cross-reactants were added to known serum pools and assayed in parallel with the base sera. No cross reaction was found. Percent cross-reactions for some of these additions are listed below in Table 5.

TABLE 5

Analyte	Concentration	Interference
CA 15-3	-	1.000
CA 125	10000 U/ml	0.001
CA 19-9	5000 U/ml	0.001
PSA	1000 ng/ml	0.026
AFP	30,000 ng/ml	ND*
CEA	5,000ng/ml	ND*
HCG	125,000mIU/ml	ND*
RF	12,500 IU/ml	0.001
Bilirubin	200 μg/ml	ND*
Hemolysis	30 μl/ml	ND*
Lipids	50 μg/ml	-0.009

15.0 REFERENCES

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Size		96(A)	192(B)
Reagent (fill)	A)	1ml set	1ml set
	B)	1 (12ml)	2 (12ml)
	C)	1 (12ml)	2 (12ml)
	D)	1 plate	2 plates
	E)	1 (20ml)	1 (20ml)
	F)	1 (50ml)	2 (50ml)
	G)	1 (12ml)	2 (12ml)
	H)	1 (8ml)	2 (8ml)

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IVD 2° 8° CE
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Please visit our website to learn more about our products and services.

Glossary of Symbols
(EN 980/ISO 15223)

IVD — In Vitro - Diagnostic Medical Device
2° 8° — Temperature Limitation Storage Condition (2-8° C)
Consult Instructions for Use
REF — Catalogue Number
Contains Sufficient Test for Σ
LOT — Batch Code
Used By (Expiration Day)
Date of Manufacturer
Manufacturer
EC REP — Authorized Rep in European Country
CE — European Conformity

Instructions for Use

TM-CA 72-4
ELISA

IVD

REF **EIA-5071**

Σ **96**

Legal Manufacturer:

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Division of DRG International, Inc
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Telefon: +49 (0)6421-17000 Fax: +49-(0)6421-1700 50
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CE

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1 INTRODUCTION

1.1 Intended Use

The **DRG TM-CA 72-4 ELISA** is an enzyme immunoassay for the quantitative *in vitro diagnostic* measurement of CA 72-4 (TAG-72) in serum and plasma.

1.2 Summary and Explanation

CA 72-4 (Cancer antigen 72-4) was originally described as an antigenic determinant recognized by B 72.3, a murine monoclonal antibody raised against a membrane extract of mammary carcinoma metastases (1). CA 72-4 was identified as a 1 MDa mucine-like Glycoprotein complex termed TAG-72 (tumor associated antigen 72) (2). The molecular weight of the TAG-72 protein is 48 kD. Elevated CA 72-4 levels in serum and plasma have been reported in various malignant diseases including carcinomas of pancreas, stomach, gall, colon, breast, ovaries, cervix and endometrium (3). The highest diagnostic sensitivities are found for carcinomas of the gastrointestinal tract and ovaries. Although some benign diseases such as rheumatic diseases or ovary cysts may also result in elevated levels of CA 72-4, clinical studies demonstrated diagnostic specificities of more than 95% for gastrointestinal and ovarian malignancies (4). There is a good correlation between CA 72-4 levels and tumor stage and size (3). CA 72-4 is the marker of choice for the therapeutic monitoring and follow-up care of gastrointestinal cancer patients. Suitable second markers are CA 19-9 or CEA. Additionally, CA 72-4 has been used as an independent marker for the therapeutic monitoring and follow-up care of ovarian cancer patients, in particular in CA 125 negative patients (3, 5).

2 PRINCIPLE OF THE TEST*

The DRG TM-CA 72-4 ELISA Kit is a solid phase enzyme-linked immunosorbent assay (ELISA) based on the sandwich principle.
The microtiter wells are coated with a monoclonal mouse antibody (Clone CC49) directed towards a unique antigenic site on a CA 72-4 molecule. An aliquot of patient sample containing endogenous CA 72-4 is incubated in the coated well with enzyme conjugate, which is an anti-CA 72-4 antibody (Clone B72.3) conjugated with horseradish peroxidase. After incubation the unbound conjugate is washed off.
The amount of bound peroxidase is proportional to the concentration of CA 72-4 in the sample.
Having added the substrate solution, the intensity of colour developed is proportional to the concentration of CA 72-4 in the patient sample.

* The antibodies used in this assay are patented by:

1. U.S. Patent No. 5,512,443, issued April 4, 1996 entitled “Second generation monoclonal antibodies having binding specificity to TAG-72 and human carcinomas and methods for employing the same” (**HHS** Reference No. E-160-1987/0-US-18)
2. Canadian Patent No. 1339980, issued August 4, 1998 entitled “Second generation monoclonal antibodies having binding specificity to TAG-72 and human carcinomas and methods for employing the same” (**HHS** Reference No. E-160-1987/0-CA-04)
3. U.S. Patent No. 4,522,918, issued June 11, 1985 (now expired) entitled “Process for Producing Monoclonal Antibodies Reactive with Human Breast Cancer” (**HHS** Reference No. E-185-1981/0-US-01)

3 WARNINGS AND PRECAUTIONS

1. This kit is for in vitro diagnostic use only. For professional use only.
2. All reagents of this test kit which contain human serum or plasma have been tested and confirmed negative for HIV I/II, HBsAg and HCV by FDA approved procedures. All reagents, however, should be treated as potential biohazards in use and for disposal.
3. Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood.
4. The microplate contains snap-off strips. Unused wells must be stored at 2 °C to 8 °C in the sealed foil pouch and used in the frame provided.
5. Pipetting of samples and reagents must be done as quickly as possible and in the same sequence for each step.
6. Use reservoirs only for single reagents. This especially applies to the substrate reservoirs. Using a reservoir for dispensing a substrate solution that had previously been used for the conjugate solution may turn solution colored. Do not pour reagents back into vials as reagent contamination may occur.
7. Mix the contents of the microplate wells thoroughly to ensure good test results. Do not reuse microwells.
8. Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
9. Allow the reagents to reach room temperature (21 °C to 26 °C) before starting the test. Temperature will affect the absorbance readings of the assay. However, values for the patient samples will not be affected.
10. Never pipet by mouth and avoid contact of reagents and specimens with skin and mucous membranes.
11. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.
12. Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of reagents or specimens may give false results.
13. Handling should be done in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.
14. Do not use reagents beyond expiry date as shown on the kit labels.
15. All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microtiter plate readers.
16. Do not mix or use components from kits with different lot numbers. It is advised not to exchange wells of different plates even of the same lot. The kits may have been shipped or stored under different conditions and the binding characteristics of the plates may result slightly different.
17. Avoid contact with *Stop Solution* containing 0.5 M H_2SO_4. It may cause skin irritation and burns.
18. Some reagents contain Proclin 300, BND and/or MIT as preservatives. In case of contact with eyes or skin, flush immediately with water.
19. TMB substrate has an irritant effect on skin and mucosa. In case of possible contact, wash eyes with an abundant volume of water and skin with soap and abundant water. Wash contaminated objects before reusing them. If inhaled, take the person to open air.
20. Chemicals and prepared or used reagents have to be treated as hazardous waste according to the national biohazard safety guideline or regulation.
21. For information on hazardous substances included in the kit please refer to Safety Data Sheets. Safety Data Sheets for this product are available upon request directly from DRG.

4 REAGENTS

4.1 Reagents provided

1. ***Microtiterwells***, 12 x 8 (break apart) strips, 96 wells;
   Wells coated with anti-CA 72-4 antibody (monoclonal).
2. ***Standard* (Standard 0-4)**, 5 vials, 0.5 mL, ready to use
   Concentration: 0, 3, 20, 50, 100 U/mL
   Contain non-mercury preservative.
3. ***Control Low & High,*** 2 vials, (lyophilized) 0.5 mL each,
   see „Reagent Preparation“
   Control values and ranges please refer to vial label or QC-Datasheet.
   Contains non-mercury preservative.
4. ***Sample Diluent***, 1 vial, 3 mL, ready to use,
   Contains non-mercury preservative.
5. ***Enzyme Conjugate 10X concentrate***, 1 vial, 1.4 mL,
   anti-CA 72-4 antibody conjugated to horseradish peroxidase;
   see „Reagent Preparation“
   Contains non-mercury preservative.
6. ***Conjugate Diluent***, 1 vial, 14 mL, ready to use
   Contains non-mercury preservative.
7. ***Substrate Solution***, 1 vial, 14 mL, ready to use,
   Tetramethylbenzidine (TMB).
8. ***Stop Solution***, 1 vial, 14 mL, ready to use,
   contains 0.5M H_2SO_4,
   Avoid contact with the stop solution. It may cause skin irritations and burns.
9. ***Wash Solution***, 1 vial, 30 mL (40X concentrated),
   see „Preparation of Reagents“.

Note: Additional *Sample Diluent* for sample dilution is available upon request.

4.2 Materials required but not provided

- A microtiter plate calibrated reader (450 ± 10 nm) (e.g. the DRG Instruments Microtiter Plate Reader).
- Calibrated variable precision micropipettes.
- Absorbent paper.
- Distilled or deionized water
- Timer
- Graph paper or software for data reduction

4.3 Storage Conditions

When stored at 2 °C to 8 °C unopened reagents will retain reactivity until expiration date. Do not use reagents beyond this date.
Opened reagents must be stored at 2 °C to 8 °C. Microtiter wells must be stored at 2 °C to 8 °C. Once the foil bag has been opened, care should be taken to close it tightly again.
Opened kits retain activity for two months if stored as described above.

4.4 Reagent Preparation

Bring all reagents and required number of strips to room temperature prior to use.

Control
Reconstitute the lyophilized content with 0.5 mL distilled water and let stand for 10 minutes in minimum.
Mix the controls several times before use.
Note: *The reconstituted controls should be apportioned and stored at -20 °C.*

Wash Solution
Add deionized water to the 40X concentrated Wash Solution.
Dilute 30 mL of concentrated Wash Solution with 1170 mL distilled water to a final volume of 1200 mL.
The diluted Wash Solution is stable for 2 weeks at room temperature.

Enzyme Conjugate
Dilute *Enzyme Conjugate* concentrate 1:10 in *Conjugate Diluent*.
Stability of the prepared Enzyme-Conjugate: 1 week at 2 °C to 8 °C in a sealed container.

Example:
If the whole plate is used, dilute 1.2 mL *Enzyme Conjugate* with 10.8 mL *Conjugate Diluent* to a total volume of 12 mL.
If the whole plate is not used at once prepare the required quantity of Enzyme Conjugate by mixing 100 µL of *Enzyme Conjugate 10X* conc. with 0.9 mL of *Conjugate Diluent* per strip (see table below):

No. of strips	*Enzyme Conjugate 10X conc*. (µL)	*Conjugate Diluent* (mL)
1	100	0.9
2	200	1.8
3	300	2.7
4	400	3.6
5	500	4.5
6	600	5.4
7	700	6.3
8	800	7.2
9	900	8.1
10	1000	9.0
11	1100	9.9
12	1200	10.8

4.5 Disposal of the Kit

The disposal of the kit must be made according to the national regulations. Special information for this product is given in the Safety Data Sheets (see chapter 13).

4.6 Damaged Test Kits

In case of any severe damage to the test kit or components, DRG has to be informed in writing, at the latest, one week after receiving the kit. Severely damaged single components should not be used for a test run. They have to be stored until a final solution has been found. After this, they should be disposed according to the official regulations.

5 SPECIMEN COLLECTION AND PREPARATION

Serum or plasma (EDTA-, Heparin- or citrate plasma) can be used in this assay.
Do not use haemolytic, icteric or lipaemic specimens.
Please note: Samples containing sodium azide should not be used in the assay.

5.1 Specimen Collection

Serum:
Collect blood by venipuncture (e.g. Sarstedt Monovette for serum), allow to clot, and separate serum by centrifugation at room temperature. Do not centrifuge before complete clotting has occurred. Patients receiving anticoagulant therapy may require increased clotting time.

Plasma:
Whole blood should be collected into centrifuge tubes containing anti-coagulant (e.g. Sarstedt Monovette with the appropriate plasma preparation) and centrifuged immediately after collection.

5.2 Specimen Storage and Preparation

Specimens should be capped and may be stored for up to 5 days at 2 °C to 8 °C prior to assaying.
Specimens held for a longer time (up to 12 months) should be frozen at -20 °C prior to assay. Thawed samples should be inverted several times prior to testing.

5.3 Specimen Dilution

If in an initial assay, a specimen is found to contain more than the highest standard, the specimens can be diluted with *Sample Diluent* and reassayed as described in Assay Procedure.
For the calculation of the concentrations this dilution factor has to be taken into account.
Example:
a) dilution 1:10: 10 µL sample + 90 µL *Sample Diluent* (mix thoroughly)
b) dilution 1:100: 10 µL dilution a) 1:10 + 90 µL *Sample Diluent* (mix thoroughly).

6 ASSAY PROCEDURE

6.1 General Remarks

- All reagents and specimens must be allowed to come to room temperature before use. All reagents must be mixed without foaming.
- Once the test has been started, all steps should be completed without interruption.
- Use new disposal plastic pipette tips for each standard, control or sample in order to avoid cross contamination
- Absorbance is a function of the incubation time and temperature. Before starting the assay, it is recommended that all reagents are ready, caps removed, all needed wells secured in holder, etc. This will ensure equal elapsed time for each pipetting step without interruption.
- As a general rule the enzymatic reaction is linearly proportional to time and temperature.

6.2 Test Procedure

Each run must include a standard curve.

1. Secure the desired number of Microtiter wells in the holder.
2. Dispense **20 µL** of each *Standard, Control* and samples with new disposable tips into appropriate wells.
3. Dispense **100 µL** freshly diluted *Enzyme Conjugate* (see "Reagent Preparation") into each well.
   Thoroughly mix for 10 seconds. It is important to have a complete mixing in this step.
4. Incubate for **120 minutes** at room temperature.
5. Briskly shake out the contents of the wells.
   Rinse the wells **5 times** with diluted *Wash Solution* (400 µL per well). Strike the wells sharply on absorbent paper to remove residual droplets.
   Important note:
   The sensitivity and precision of this assay is markedly influenced by the correct performance of the washing procedure!
6. Add **100 µL** of *Substrate Solution* to each well.
7. Incubate for **30 minutes** at room temperature.
8. Stop the enzymatic reaction by adding **100 µL** of *Stop Solution* to each well.
9. Determine the absorbance (OD) of each well at **450 ± 10 nm** with a microtiter plate reader.
   It is recommended that the wells be read **within 10 minutes** after adding the *Stop Solution*.

6.3 Calculation of Results

1. Calculate the average absorbance values for each set of standards, controls and patient samples.
2. Manual method: Using linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.
4. Automated method: The results in the Instructions for Use have been calculated automatically using a 4 Parameter curve fit. (4 Parameter Rodbard or 4 Parameter Marquardt are the preferred methods.)
   Other data reduction functions may give slightly different results.
5. The concentration of the samples can be read directly from this standard curve. Samples with concentrations higher than that of the highest standard have to be further diluted or reported as > 100 U/mL. For the calculation of the concentrations this dilution factor has to be taken into account.

6.3.1 Example of Typical Standard Curve

The following data is for demonstration only and **cannot** be used in place of data generations at the time of assay.

Standard	Optical Units (450 nm)
Standard 0 (0 U/mL)	0.08
Standard 1 (3 U/mL)	0.19
Standard 2 (20 U/mL)	0.59
Standard 3 (50 U/mL)	1.16
Standard 4 (100 U/mL)	2.02

7 EXPECTED NORMAL VALUES

It is strongly recommended that each laboratory should determine its own normal and abnormal values.

In a study conducted with apparently normal healthy adults, using the DRG TM-CA 72-4 ELISA the following values are observed:

Population	Valid N	Median	Mean	5^{th} - 95^{th} Percentile
apparently healthy subjects	65	0.72 U/mL	0.86 U/mL	0 U/mL - 2.68 U/mL

The results are in good agreement with published cut-offs between 4 U/mL - 6 U/mL (Reference/Literature 3-6).

The results alone should not be the only reason for any therapeutic consequences. The results should be correlated to other clinical observations and diagnostic tests.

8 QUALITY CONTROL

Good laboratory practice requires that controls be run with each calibration curve. A statistically significant number of controls should be assayed to establish mean values and acceptable ranges to assure proper performance.
It is recommended to use control samples according to state and federal regulations. The use of control samples is advised to assure the day to day validity of results. Use controls at both normal and pathological levels.
The controls and the corresponding results of the QC-Laboratory are stated in the QC certificate added to the kit. The values and ranges stated on the QC sheet always refer to the current kit lot and should be used for direct comparison of the results.
It is also recommended to make use of national or international Quality Assessment programs in order to ensure the accuracy of the results.
Employ appropriate statistical methods for analysing control values and trends. If the results of the assay do not fit to the established acceptable ranges of control materials patient results should be considered invalid.
In this case, please check the following technical areas: Pipetting and timing devices; photometer, expiration dates of reagents, storage and incubation conditions, aspiration and washing methods.
After checking the above mentioned items without finding any error contact your distributor or DRG directly.

9 PERFORMANCE CHARACTERISTICS

9.1 Assay Dynamic Range

The range of the assay is between 0.79 U/mL – 100 U/mL.

9.2 Specificity of Antibodies (Cross Reactivity)

No cross reactivity was observed with related proteins.

9.3 Sensitivity

The analytical sensitivity was calculated by adding 2 standard deviations to the mean of 20 replicate analyses of *Standard 0* and was found to be 0.79 U/mL.

9.4 Reproducibility

9.4.1 Intra Assay

The within assay variability is shown below:

Sample	n	Mean (U/mL)	CV (%)
1	20	1.4	2.2
2	20	1.6	1.6
3	20	1.6	2.4

9.4.2 Inter Assay

The between assay variability is shown below:

Sample	n	Mean (U/mL)	CV (%)
1	40	10.1	4.4
2	40	18.9	4.8
3	40	29.6	4.2

9.5 Recovery

Samples have been spiked by adding CA 72-4 solutions with known concentrations in a 1:1 ratio.
The expected values were calculated by addition of half of the values determined for the undiluted samples and half of the values of the known solutions. The % Recovery has been calculated by multiplication of the ratio of the measurements and the expected values with 100.

		Sample 1	Sample 2	Sample 3
Concentration [U/mL]		3.6	8.1	9.4
Average Recovery [%]		99.3	98.2	98.8
Range of Recovery [%]	from	96.6	92.5	88.2
	to	102.1	105.8	106.8

9.6 Linearity

		Sample 1	Sample 2	Sample 3
Concentration [U/mL]		51.0	94.0	10.0
Average Recovery [%]		91.2	108.5	97.8
Range of Recovery [%]	from	86.3	106.4	86.0
	to	99.6	112.3	112.0

10 LIMITATIONS OF USE

Reliable and reproducible results will be obtained when the assay procedure is performed with a complete understanding of the package insert instruction and with adherence to good laboratory practice.
Any improper handling of samples or modification of this test might influence the results.

10.1 Interfering Substances

Haemoglobin (up to 4 mg/mL), Bilirubin (up to 0.5 mg/mL) and Triglyceride (up to 7.5 mg/mL) have no influence on the assay results.
Triglycerides > 7.5 mg/mL will result in decreased values.

The assay contains reagents to minimize interference of HAMA and heterophilic antibodies. However, extremely high titers of HAMA or heterophilic antibodies may interfere with the test results.

10.2 Drug Interferences

Until today no substances (drugs) are known to us, which have an influence to the measurement of CA 72-4 in a sample.

10.3 High-Dose-Hook Effect

No hook effect was observed in this test up to 6,400 U/mL of CA 72-4.

11 LEGAL ASPECTS

11.1 Reliability of Results

The test must be performed exactly as per the manufacturer's instructions for use. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable national standards and/or laws. This is especially relevant for the use of control reagents. It is important to always include, within the test procedure, a sufficient number of controls for validating the accuracy and precision of the test.
The test results are valid only if all controls are within the specified ranges and if all other test parameters are also within the given assay specifications. In case of any doubt or concern please contact DRG.

11.2 Therapeutic Consequences

Therapeutic consequences should never be based on laboratory results alone even if all test results are in agreement with the items as stated under point 11.1. Any laboratory result is only a part of the total clinical picture of a patient. Only in cases where the laboratory results are in acceptable agreement with the overall clinical picture of the patient should therapeutic consequences be derived.
The test result itself should never be the sole determinant for deriving any therapeutic consequences.

11.3 Liability

Any modification of the test kit and/or exchange or mixture of any components of different lots from one test kit to another could negatively affect the intended results and validity of the overall test. Such modification and/or exchanges invalidate any claim for replacement.
Claims submitted due to customer misinterpretation of laboratory results subject to point 11.2. are also invalid. Regardless, in the event of any claim, the manufacturer's liability is not to exceed the value of the test kit. Any damage caused to the test kit during transportation is not subject to the liability of the manufacturer.

12 REFERENCES / LITERATURE

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1 EINLEITUNG

Der **DRG TM-CA 72-4 ELISA** wird zur quantitativen Bestimmung von CA 72-4 in Serum und Plasma eingesetzt.
Nur für In-vitro Diagnostik.

2 TESTPRINZIP*

Der DRG TM-CA 72-4 ELISA ist ein Festphasen-Enzymimmunoassay, der auf der Sandwichtechnik basiert.
Die Wells der Mikrotiterplatten sind mit einem monoklonalen Antikörper (Clone CC49) beschichtet, der gegen eine definierte Antikörper-Bindungsstelle des CA 72-4-Moleküls gerichtet ist.
Die Proben werden in die beschichteten Wells gegeben und mit einem Enzymkonjugat inkubiert. Das Konjugat enthält einen monoklonalen anti-CA 72-4-Antikörper (Clone B72.3), der mit Meerrettichperoxidase konjugiert ist. Es wird ein Sandwichkomplex gebildet.
Das nicht gebundene Konjugat wird durch Waschen der Wells entfernt. Anschließend wird die Substratlösung zugegeben und die Farbentwicklung nach einer definierten Zeit gestoppt. Die Intensität der gebildeten Farbe ist proportional der CA 72-4-Konzentration in der Probe. Die Extinktion wird bei 450 nm mit einem Mikrotiterplattenleser gemessen.

* Die in diesem Assay eingesetzten Antikörper sind durch folgende Patente geschützt:

1. U.S. Patent No. 5,512,443, issued April 4, 1996 entitled “Second generation monoclonal antibodies having binding specificity to TAG-72 and human carcinomas and methods for employing the same” (**HHS** Reference No. E-160-1987/0-US-18)
2. Canadian Patent No. 1339980, issued August 4, 1998 entitled “Second generation monoclonal antibodies having binding specificity to TAG-72 and human carcinomas and methods for employing the same” (**HHS** Reference No. E-160-1987/0-CA-04)
3. U.S. Patent No. 4,522,918, issued June 11, 1985 (now expired) entitled “Process for Producing Monoclonal Antibodies Reactive with Human Breast Cancer” (**HHS** Reference No. E-185-1981/0-US-01)

3 VORSICHTSMAßNAHMEN

- Dieser Kit ist nur zum in vitro diagnostischen Gebrauch geeignet.
- Nur die gültige, im Testkit enthaltene, Arbeitsanleitung verwenden.
- Informationen zu im Kit enthaltenen gefährlichen Substanzen entnehmen Sie bitte dem Materialsicherheitsdatenblatt.
- Alle Bestandteile dieses Testkits, die humanes Serum oder Plasma enthalten, wurden mit FDA-geprüften Methoden auf HIV I/II, HbsAg und HCV getestet und als negativ bestätigt. Jedoch sollten alle Bestandteile im Umgang und bei der Entsorgung wie mögliche Gefahrenstoffe betrachtet werden.
- Der Kontakt mit der *Stop Solution* sollte vermieden werden, da sie 0.5 M H_2SO_4 enthält. Schwefelsäure kann Hautreizungen und Verbrennungen verursachen.
- Nicht mit dem Mund pipettieren und den Kontakt von Kitbestandteilen und Proben mit Haut und Schleimhäuten vermeiden.
- In den Bereichen, in denen Proben oder Kitbestandteile verwendet werden, nicht rauchen, essen oder Kosmetika verwenden.
- Beim Umgang mit Proben oder Reagenzien Einweg-Latexhandschuhe tragen. Die Verunreinigung von Reagenzien oder Proben mit Mikroben kann zu falschen Ergebnissen führen.
- Der Gebrauch sollte gemäß der Vorschriften einer entsprechenden nationalen Gefahrenstoff-Sicherheitsrichtlinie erfolgen.
- Reagenzien nicht nach dem auf dem Kit-Etikett angegebenen Verfallsdatum verwenden.
- Alle im Kit-Protokoll angegebenen Mengen müssen genau eingehalten werden. Optimale Ergebnisse können nur durch Verwendung kalibrierter Pipetten und Mikrotiterplatten-Lesegeräte erreicht werden.
- Komponenten von Kits mit unterschiedlichen Lotnummern nicht untereinander vertauschen. Es wird empfohlen, keine Wells von verschiedenen Platten zu verwenden, auch nicht, wenn es sich um das gleiche Lot handelt. Die Kits können unter anderen Bedingungen gelagert oder versendet worden sein, so dass die Bindungscharakteristik der Platten leicht unterschiedlich ausfällt.
- Chemikalien und zubereitete oder bereits benutzte Reagenzien müssen gemäß den nationalen Gefahrenstoffvorschriften wie gefährlicher Abfall behandelt werden.
- Sicherheitsdatenblätter für dieses Produkt sind auf Anfrage direkt von der Firma DRG Instruments GmbH erhältlich.

4 BESTANDTEILE DES KITS

4.1 Kitinhalt

1. ***Microtiterwells***, 96 Wells, 12 x 8 Wells (einzeln brechbar);
   Mit anti- CA 72-4 -Antikörper (monoklonal) beschichtet.
2. ***Standard (Standard 0-4)***, 5 Fläschchen, je 0,5 mL, gebrauchsfertig;
   Konzentrationen: 0; 3, 20, 50, 100 U/mL.
   Enthält quecksilberfreies Konservierungsmittel.
3. ***Control Low & High*** (Kontrolle), 2 Fläschchen (lyophilisiert), je 0,5 mL;
   Siehe „Vorbereitung der Reagenzien“.
   Kontrollwerte und –bereiche entnehmen Sie bitte dem Fläschchenetikett oder dem QC-Datenblatt.
   Enthält quecksilberfreies Konservierungsmittel.
4. ***Sample Diluent*** (Probenverdünnungsmedium), 1 Fläschchen, 3 mL, gebrauchsfertig;
   Enthält quecksilberfreies Konservierungsmittel.
5. ***Enzyme Conjugate*** (Enzymkonjugat)***10X*** **konzentriert**, 1 Fläschchen, 1,4 mL;
   Anti- CA 72-4 -Antikörper, mit Meerrettichperoxidase konjugiert ,
   siehe „Vorbereitung der Reagenzien“.
   Enthält quecksilberfreies Konservierungsmittel.
6. ***Conjugate Diluent*** (Konjugatverdünnungsmedium), 1 Fläschchen, 14 mL, gebrauchsfertig.
   Enthält quecksilberfreies Konservierungsmittel.
7. ***Substrate Solution*** (Substratlösung), 1 Fläschchen, 14 mL, gebrauchsfertig;
   Substratlösung TMB.
8. ***Stop Solution*** (Stopplösung), 1 Fläschchen, 14 mL, gebrauchsfertig;
   enthält 0,5M H_2SO_4,
   Kontakt mit der Stopplösung vermeiden! Kann Hautreizungen und Verbrennungen verursachen.
9. ***Wash Solution*** (Waschlösung), 1 Fläschchen, 30 mL, **40X** konzentriert;
   Siehe „Vorbereitung der Reagenzien“.

Anmerkung: Zusätzliches *Sample Diluent* zur Probenverdünnung ist auf Anfrage erhältlich.

4.2 Nicht im Kit enthaltene aber erforderliche Geräte und Materialien

- Kalibriertes Mikrotiterplattenlesegerät mit 450 ± 10 nm Filter), (z.B. das DRG Instruments Mikrotiterplattenlesegerät)
- Kalibrierte variable Präzisions-Mikropipette
- Saugfähiges Papier
- Aqua dest.

4.3 Lagerung und Haltbarkeit des Kits

Die ungeöffneten Reagenzien behalten bei Lagerung um 2 °C - 8 °C ihre Reaktivität bis zum Verfallsdatum. Nach dem Verfallsdatum die Reagenzien nicht mehr verwenden.
Nach dem Öffnen sollten alle Reagenzien bei 2 °C - 8 °C gelagert werden.
Die Mikrotiterwells sollten bei 2 °C - 8 °C gelagert werden. Der einmal geöffnete Folienbeutel sollte stets sehr sorgfältig wieder verschlossen werden.
Unter den beschriebenen Lagerbedingungen behalten geöffnete Kits zwei Monate ihre Reaktivität.

4.4 Vorbereitung der Reagenzien

Alle Reagenzien sowie die benötigte Anzahl von Wells sollen vor dem Gebrauch auf Raumtemperatur gebracht werden.

Control
Rekonstituieren Sie den lyophilisierten Inhalt der Fläschchen mit 0,5 mL destilliertem Wasser und lassen Sie die Fläschchen mindestens 10 Minuten ruhen. Vor Gebrauch die Kontrollen mehrmals vorsichtig schütteln.
Achtung: Die rekonstituierten Kontrollen müssen portioniert und bei -20 °C eingefroren werden.

Wash Solution
Die 40-fach konzentrierte Waschlösung (30 mL) mit 1170 mL destilliertem Wasser auf ein Gesamtvolumen von 1200 mL verdünnen.
Die verdünnte Waschlösung ist bei Raumtemperatur für 2 Wochen stabil.

Enzyme Conjugate
Verdünnen Sie das *Enzymkonjugat*-Konzentrat 1:10 mit *Conjugate Diluent*.
Stabilität der Enzymkonjugat-Arbeitslösung: 1 Woche bei 2 °C - 8 °C in einem verschlossenen Behälter.

Beispiel:
Wird die ganze Platte verwendet, verdünnen Sie 1,2 mL *Enzyme Conjugate* mit 10,8 mL *Conjugate Diluent* (Gesamtvolumen 12 mL).
Wird nur ein Teil der Platte benötigt, setzen Sie nur das erforderliche Volumen des Konjugates an. Verdünnen Sie dafür pro Streifen 100 µL *Enzyme Conjugate*-Konzentrat mit 0,9 mL *Conjugate Diluent* (siehe Tabelle):

Anzahl der Streifen	*Enzyme Conjugate 10X* konz. (µL)	*Conjugate Diluent* (mL)
1	100	0,9
2	200	1,8
3	300	2,7
4	400	3,6
5	500	4,5
6	600	5,4
7	700	6,3
8	800	7,2
9	900	8,1
10	1000	9,0
11	1100	9,9
12	1200	10,8

4.5 Entsorgung des Kits

Die Entsorgung des Kits muss gemäß den nationalen gesetzlichen Vorschriften erfolgen. Spezielle Informationen für dieses Produkt finden Sie im Sicherheitsdatenblatt, Kapitel 13.

4.6 Beschädigte Testkits

Im Falle einer starken Beschädigung des Testkits oder der Komponenten muss die Firma DRG in schriftlicher Form spätestens eine Woche nach Erhalt des Kits informiert werden. Stark beschädigte Einzelkomponenten sollten nicht für den Testlauf verwendet werden. Sie müssen gelagert werden bis eine endgültige Lösung gefunden wurde. Danach sollten Sie gemäß den offiziellen Richtlinien entsorgt werden.

5 PROBENVORBEREITUNG

Serum oder Plasma (EDTA-, Heparin- oder Citratplasma) kann in diesem Test als Probenmaterial eingesetzt werden. Lipämische, ikterische und/oder stark hämolysierte Proben sollten nicht verwendet werden.
Achtung: Proben, die Natriumazid enthalten, sollten nicht verwendet werden.

5.1 Probenentnahme

Serum:
Blut durch Venenpunktion entnehmen (z.B. mit Sarstedt Monovette für Serum), gerinnen lassen und das Serum durch Zentrifugation bei Raumtemperatur abtrennen. Vor der Zentrifugation muss die Gerinnung vollständig abgeschlossen sein. Bei Patienten, die Antikoagulantien erhalten, kann die Gerinnungszeit länger dauern.

Plasma:
Die Blutentnahme erfolgt mit Röhrchen, die ein Antikoagulanz enthalten (z.B.: Sarstedt Monovette – mit entsprechender Plasma-Präparierung). Das Plasma wird als Überstand nach einer Zentrifugation gewonnen.

5.2 Probenaufbewahrung

Proben sollten stets gut verschlossen sein und können vor Testbeginn bis zu 5 Tage bei 2 °C - 8 °C gelagert werden. Für eine längere Aufbewahrung (bis zu 12 Monaten) sollten die Proben eingefroren bei -20 °C bis zum Testbeginn gelagert werden. Nur einmal einfrieren. Aufgetaute Proben sollten vor Testbeginn vorsichtig durchmischt werden, ohne Schaumbildung.

5.3 Probenverdünnung

Wenn in einem ersten Testdurchlauf bei einer Probe eine Konzentration höher als der höchste Standard gefunden wird, kann diese Probe mit *Sample Diluent* weiter verdünnt und nochmals bestimmt werden. Die Verdünnung muss jedoch bei der Berechnung der Konzentration beachtet werden.
Beispiel:
a) Verdünnung 1:10: 10 µL Probe + 90 µL *Sample Diluent* gründlich mischen)
b) Verdünnung 1:100: 10 µL Verdünnung a) 1:10 + 90 µL *Sample Diluent* (gründlich mischen).

6 TESTDURCHFÜHRUNG

6.1 Allgemeine Hinweise

Alle Reagenzien und Proben müssen vor Gebrauch auf Raumtemperatur gebracht und gut durchgemischt werden. Dabei sollte Schaumbildung vermieden werden.
Wenn die Testdurchführung einmal begonnen wurde, muss sie ohne Unterbrechung zu Ende geführt werden.
Für jeden Standard, jede Kontrolle oder Probe eine neue Plastikspitze verwenden, um Verschleppungen zu vermeiden.
Die Optische Dichte ist abhängig von Inkubationszeit und Temperatur. Deshalb ist es notwendig, vor Beginn der Testdurchführung alle Reagenzien in einen arbeitsbereiten Zustand zu bringen, die Deckel der Fläschchen zu öffnen, alle benötigten Wells in den Halter zu setzen. Nur eine solche Vorbereitung garantiert gleiche Zeiten für jeden Pipettiervorgang ohne Pausen.
Als generelle Regel gilt, dass die enzymatische Reaktion linear proportional zu Zeit und Temperatur ist.

6.2 Testdurchführung

Jeder Lauf muss eine Standardkurve beinhalten.

1. Die benötigte Anzahl Wells in der Halterung befestigen.
2. **Je 20 µL** *Standards, Control* und Proben mit neuen Plastikspitzen in die entsprechenden Wells geben.
3. **100 µL** frisch angesetztes *Enzyme-Conjugate* (siehe „Vorbereitung der Reagenzien“) in jedes Well geben. Für 10 Sekunden gut schütteln. Es ist sehr wichtig, in diesem Schritt eine komplette Durchmischung zu erreichen.
4. **120 Minuten** bei Raumtemperatur inkubieren.
5. Den Inhalt der Wells kräftig ausschütteln. Wells 5-mal mit verdünnter *Wash Solution* (400 µL/Well) waschen. Verbleibende Flüssigkeit durch Ausklopfen der Wells auf saugfähigem Papier entfernen.
   Achtung:
   Die Sensitivität und Präzision dieses Assays wird erheblich beeinflusst von der korrekten Durchführung des Waschschrittes!
6. **100 µL** *Substrate Solution* in jedes Well geben.
7. **30 Minuten** bei Raumtemperatur inkubieren.
8. Die enzymatische Reaktion durch Zugabe von **100 µL** *Stop Solution* in jedes Well abstoppen.
9. Die Optische Dichte bei **450 ± 10 nm** mit einem Mikrotiterplatten-Lesegerät innerhalb von **10 Minuten** nach Zugabe der *Stop Solution* bestimmen.

6.3 Ergebnisermittlung

1. Die durchschnittlichen Werte der Optischen Dichte (OD) für jedes Set von Standards, Controls und Patientenproben bestimmen.
2. Eine Standardkurve ermitteln durch Auftragen der mittleren Optischen Dichte jedes Standards gegen die Konzentration, wobei der OD-Wert auf der vertikalen (Y) Achse und die Konzentration auf der horizontalen (X) Achse eingetragen wird.
3. Unter Verwendung der mittleren OD wird für jede Probe die entsprechende Konzentration aus der Standardkurve ermittelt.
4. Automatische Methode: Die in der Gebrauchsanweisung angegebenen Werte wurden automatisch mit Hilfe der 4 Parameter Gleichung bestimmt. (4 Parameter Rodbard oder 4 Parameter Marquardt sind die bevorzugten Methoden.) Andere Auswertungsfunktionen können leicht abweichende Werte ergeben.
5. Die Konzentration der Proben kann direkt von der Standardkurve abgelesen werden. Proben, die eine höhere Konzentration als die des höchsten Standards enthalten, müssen verdünnt werden. Dieser Verdünnungsfaktor muss bei der Berechnung der Konzentration beachtet werden.

6.3.1 Beispiel für eine Standardkurve

Nachfolgend wird ein typisches Beispiel für eine Standardkurve mit dem DRG ELISA gezeigt. Diese Werte sollten **nicht** zur Berechnung von Patientendaten verwendet werden.

Standard	Optische Dichte (450nm)
Standard 0 (0 U/mL)	0,05
Standard 1 (3 U/mL)	0,14
Standard 2 (20 U/mL)	0,65
Standard 3 (50 U/mL)	1,25
Standard 4 (100 U/mL)	2,04

7 ERWARTETE WERTE

Es wird empfohlen, dass jedes Labor seine eigenen normalen und abnormalen Werte ermittelt.

Die Normwertermittlung wurde durchgeführt mit 65 gesunden Individuen.

Probanden	Anzahl	Median	Mittelwert	5. - 95. Perzentile
gesunde Männer und Frauen	65	0,72 U/mL	0,86 U/mL	0 U/mL - 2,68 U/mL

Die Ergebnisse stimmen gut mit Literaturangaben zum Grenzwertbereich (cut-off) für gesunde Personen von 4 U/mL - 6 U/mL überein (Reference/Literature 3-6)

8 QUALITÄTS-KONTROLLE

Es wird empfohlen, die Kontrollproben gemäß den nationalen gesetzlichen Bestimmungen einzusetzen. Durch die Verwendung von Kontrollproben wird eine Tag-zu-Tag Überprüfung der Ergebnisse erzielt. Es sollten Kontrollen sowohl mit normalem als auch pathologischem Level eingesetzt werden.
Die Kontrollen mit den entsprechenden Ergebnissen des QC-Labors sind im QC-Zertifikat, das dem Kit beiliegt, aufgeführt. Die im QC-Blatt angegebenen Werte und Bereiche beziehen sich stets auf die aktuelle Kitcharge und sollten zum direkten Vergleich der Ergebnisse verwendet werden.
Es wird ebenfalls empfohlen, an nationalen oder internationalen Qualitätssicherungs-Programmen teilzunehmen, um die Genauigkeit der Ergebnisse zu sichern.
Es sollten geeignete statistische Methoden zur Analyse von Kontroll-Werten und Trends angewendet werden. Wenn die Ergebnisse des Assays nicht mit den angegebenen Akzeptanzbereichen des Kontrollmaterials übereinstimmen, sollten die Patientenergebnisse als ungültig eingestuft werden.
In diesem Fall überprüfen Sie bitte die folgenden Bereiche: Pipetten und Zeitnehmer, Photometer, Verfallsdatum der Reagenzien, Lagerungs- und Inkubationsbedingungen, Absaug- und Waschmethode.
Sollten Sie nach Überprüfung der vorgenannten Bereiche keinen Fehler erkannt haben, setzen Sie sich bitte mit Ihrem Lieferanten oder direkt mit der Firma DRG in Verbindung.

9 ASSAY CHARACTERISTIKA

9.1 Messbereich

Der Messbereich des Testes liegt zwischen 0,79 – 100 U/mL.

9.2 Spezifität der Antikörper (Kreuzreaktivität)

Die Daten entnehmen Sie bitte der ausführlichen englischen Arbeitsanleitung.

9.3 Analytische Sensitivität

Die analytische Sensitivität, definiert als Mittelwert plus der zweifachen Standardabweichung des *Standards 0* (n = 20), beträgt < 0,79 U/mL.

Die Daten zu:

9.4 Reproduzierbarkeit (Präzision)

9.5 Wiederfindung

9.6 Linearität

entnehmen Sie bitte der ausführlichen englischen Arbeitsanleitung.

10 GRENZEN DES TESTS

Jede unsachgemäße Behandlung von Proben oder Modifikationen dieses Tests können die Ergebnisse beeinflussen.

10.1 Interferenzen

Hämoglobin (bis zu 4 mg/mL), Bilirubin (bis zu 0.5 mg/mL) und Triglyceride (bis zu 7,5 mg/mL) haben keinen Einfluss auf das Testergebnis.
Triglyceride > 7.5 mg/mL führen zu erniedrigten Werten.

Der Test enthält Reagenzien, um Interferenzen mit HAMA oder heterophilen Antikörpern zu minimieren. Dennoch ist es möglich, dass ein sehr hoher Titer von HAMA oder heterophilen Antikörpern das Testergebnis beeinflusst.

10.2 Beeinflussung durch Medikamente

Uns sind bislang keine Stoffe (Medikamente) bekannt geworden, deren Einnahme die Messung des CA 72-4-Gehaltes der Probe beeinflussen würde.

10.3 High-Dose-Hook Effekt

Ein Hook Effekt tritt bei Proben mit bis zu 6.400 U/mL CA 72-4 nicht auf.

11 RECHTLICHE GRUNDLAGEN

11.1 Zuverlässigkeit der Ergebnisse

Der Test muss exakt gemäß der Testanleitung des Herstellers abgearbeitet werden. Darüber hinaus muss der Benutzer sich strikt an die Regeln der GLP (Good Laboratory Practice) oder andere eventuell anzuwendende Regeln oder nationale gesetzliche Vorgaben halten. Dies betrifft besonders den Gebrauch der Kontrollreagenzien. Es ist sehr wichtig, bei der Testdurchführung stets eine ausreichende Anzahl Kontrollen zur Überprüfung der Genauigkeit und Präzision mitlaufen zu lassen.
Die Testergebnisse sind nur gültig, wenn alle Kontrollen in den vorgegebenen Bereichen liegen, und wenn alle anderen Testparameter die vorgegebenen Spezifikationen für diesen Assay erfüllen. Wenn Sie bezüglich eines Ergebnisses Zweifel oder Bedenken haben, setzen Sie sich bitte mit der Firma DRG in Verbindung.

11.2 Therapeutische Konsequenzen

Therapeutische Konsequenzen sollten keinesfalls nur aufgrund von Laborergebnissen erfolgen, selbst dann nicht, wenn alle Testergebnisse mit den in 11.1. genannten Voraussetzungen übereinstimmen. Jedes Laborergebnis ist nur ein Teil des klinischen Gesamtbildes eines Patienten.
Nur in Fällen, in denen die Laborergebnisse in akzeptabler Übereinstimmung mit dem allgemeinen klinischen Bild des Patienten stehen, sollten therapeutische Konsequenzen eingeleitet werden.
Das Testergebnis allein sollte niemals als alleinige Grundlage für die Einleitung therapeutischer Konsequenzen dienen.

11.3 Haftung

Jegliche Veränderungen des Testkits und/oder Austausch oder Vermischung von Komponenten unterschiedlicher Chargen von einem Testkit zu einem anderen, können die gewünschten Ergebnisse und die Gültigkeit des gesamten Tests negativ beeinflussen. Solche Veränderungen und/oder Austausch haben den Ausschluss jeglicher Ersatzansprüche zur Folge.
Reklamationen, die aufgrund von Falschinterpretation von Laborergebnissen durch den Kunden gemäß Punkt 11.2. erfolgen, sind ebenfalls abzuweisen. Im Falle jeglicher Reklamation ist die Haftung des Herstellers maximal auf den Wert des Testkits beschränkt. Jegliche Schäden, die während des Transports am Kit entstanden sind, unterliegen nicht der Haftung des Herstellers.

12 REFERENZEN / LITERATUR

Angaben zu den Referenzen entnehmen Sie bitte der ausführlichen englischen Arbeitsanleitung.

1 INTRODUZIONE

Il test immuno-enzimatico **DRG TM-CA 72-4 ELISA** contiene materiale per la determinazione quantitativa di CA 72-4 (TAG-72)in siero e plasma.
Questo test kit è adatto soltanto per l'uso diagnostico.

2 PRINCIPIO DEL TEST*

Il test kit DRG TM-CA 72-4 ELISA è un test immunologico in fase solida con enzimi ancorati su un substrato (ELISA) basato sul principio sandwich.
I micropozzetti sono ricoperti con un anticorpo monoclinale (Clone CC49) diretto contro un unico sito antigenico su una molecola CA 72-4. Un'aliquota di un campione di paziente contenente CA 72-4 endogeno/a viene incubato nel pozzetto ricoperto dell'enzima coniugato, che è un anticorpo anti- CA 72-4 (Clone B72.3) coniugato alla perossidasi di rafano. Dopo l'incubazione il coniugato non legato è eliminato attraverso lavaggi.
La quantità della perossidasi legata è proporzionale alla concentrazione CA 72-4 nel campione.
Dopo l'aggiunta della soluzione substrato l'intensità del colore sviluppato è proporzionale alla concentrazione di CA 72-4 nel campione del paziente.

* Anticorpi utilizzati in questo test sono protetti da brevetti:

1. U.S. Patent No. 5,512,443, issued April 4, 1996 entitled "Second generation monoclonal antibodies having binding specificity to TAG-72 and human carcinomas and methods for employing the same" (**HHS** Reference No. E-160-1987/0-US-18)
2. Canadian Patent No. 1339980, issued August 4, 1998 entitled "Second generation monoclonal antibodies having binding specificity to TAG-72 and human carcinomas and methods for employing the same" (**HHS** Reference No. E-160-1987/0-CA-04)
3. U.S. Patent No. 4,522,918, issued June 11, 1985 (now expired) entitled "Process for Producing Monoclonal Antibodies Reactive with Human Breast Cancer" (**HHS** Reference No. E-185-1981/0-US-01)

3 PRECAUZIONI

- Questo kit è adatto soltanto per l'uso diagnostico in vitro.
- Si prega di usare la versione valida dell'inserto del pacco a disposizione con il kit.
- Informazioni su sostanze pericolose contenute nel kit sono riportate nel regolamento di sicurezza.
- Tutti i componenti del kit che contengono siero o plasma umano sono controllati e confermati negativi per la presenza di HIV I/II, HbsAg e HCV con metodi conformi alle norme FDA. Ciononostante tutti i componenti dovrebbero essere trattati come potenziali sostanze nocive nella manutenzione e nello smaltimento.
- Il contatto con la *Stop Solution* dovrebbe essere evitato perché contiene 0.5 M H_2SO_4. L'acido solforico può provocare irritazioni cutanee e ustioni.
- Non pipettare con la bocca ed evitare il contatto con componenti del kit con la pelle o con le mucose.
- Nelle aree in cui il test viene utilizzato non fumare, mangiare, bere o fare uso di prodotti cosmetici.
- Nella manutenzione dei campioni o reagenti del kit portare guanti di latex monouso. La contaminazione dei reagenti o dei campioni con microbi può dare risultati falsi.
- L'utilizzo dovrebbe avvenire secondo regole che seguono le rispettive norme di sicurezza nazionali sulle sostanze nocive.
- Non utilizzare i reagenti dopo la scadenza indicata sul kit.
- Ogni indicazione sulla quantità indicata del protocollo del kit deve essere accuratamente seguito. Risultati ottimali possono essere ottenuti soltanto con l'uso di pipette calibrate e spettrofotometro calibrato.
- Componenti del kit con numeri di lotto diversi non devono essere combinati. È consigliabile di non utilizzare pozzetti di piastre diversi, anche se si tratta dello stesso lotto. I kit potrebbero essere stati magazzinati o spediti a condizioni diverse, cosicché le caratteristiche di legame potrebbero divergere leggermente.
- I componenti chimici e reagenti preparati o già utilizzati devono essere trattati e smaltiti secondo le norme di sicurezza nazionali sulle sostanze nocive.
- I regolamenti di sicurezza di questo prodotto possono essere richiesti direttamente dalla ditta DRG Instruments GmbH.

4 COMPONENTI DEL KIT

4.1 Contenuto del kit

1. ***Microtiterwells*** (Micropozetti), 12 x 8 file (separatamente staccabili), 96 pozzetti;
   Pozzetti ricoperti con l'anti-CA 72-4 anticorpo (monoclinale)
2. ***Standard (Standard 0-4)***, 5 flaconi, 0,5 mL; pronto all'uso
   Concentrazione: 0; 3, 20, 50, 100 U/mL.
   Contiene conservante senza mercurio.
3. ***Control Low & High*** (Controllo), 2 flaconi (liofillizzato), 0,5 mL
   vedi „preparazione dei reagenti"
   I valori dei controlli sono indicati sull'etichetta dei flaconi o sulla descrizione QC.
   Contiene conservante senza mercurio.
4. ***Sample Diluent*** (Diluente dei campioni), 1 flacone, 3 mL, pronto all'uso
   Contiene conservante senza mercurio.
5. ***Enzyme Conjugate Concentrate 10X*** (tracciante enzimatico concentrato 10X), 1 flacone, 1.4 mL,
   anti- CA 72-4 anticorpo coniugato alla perossidasi di rafano
   vedi „preparazione dei reagenti".
   Contiene conservante senza mercurio.
6. ***Conjugate Diluent*** (Diluente del tracciante), 1 flacone, 14 mL, pronto all'uso
   Contiene conservante senza mercurio.
7. ***Substrate Solution*** (Soluzione di substrato), 1 flacone, 14 mL, pronto all'uso;
   TMB (benzidine tetrametilico).
8. ***Stop Solution*** (Soluzione d'arresto), 1 flacone, 14 mL, pronto all'uso;
   contiene 0.5M H_2SO_4.
   Evitare il contatto con la soluzione d'arresto. Può causare irritazioni cutanee e ustioni.
9. ***Wash Solution*** (Soluzione di lavaggio), 1 flacone, 30 mL (concentrata 40X);
   vedi „preparazione dei reagenti".

Nota: Ulteriore *Sample Diluent* può essere richiesto alla ditta.

4.2 Materiali richiesti ma non contenuti nel kit

- Uno spettrofotometro calibrato per micropozzetti (450±10 nm) (p.es. il DRG Instruments Microtiterplate Reader).
- Micropipette calibrate di precisione a volume variabile.
- Carta assorbente.
- Acqua distillata.

4.3 Magazzinaggio e stabilità del kit

A 2 °C - 8 °C i reagenti non aperti rimangono reattivi fino alla data di scadenza indicata. Non usare reagenti oltre questa data.
Tutti i reagenti aperti devono essere magazzinati a 2 °C - 8 °C. I micropozzetti devono essere magazzinati a 2 °C - 8 °C. Una volta aperti i pacchi, questi devono essere richiusi accuratamente.
Test kits aperti rimangono attivi per due mesi se magazzinati alle condizioni sopra descritte.

4.4 Preparazione dei reagenti

Prima dell'uso portare tutti i reagenti e il numero necessario di pozzetti a temperatura ambiente.

Control
Ricostituire il contenuto liofillizzato con 0,5 mL acqua distillata e far riposare per almeno 10 minuti. Mescolare alcune volte prima dell'uso.
Nota: *I controlli ricostituiti dovrebbe essere aliquotati e magazzinati a -20 °C.*

Wash Solution
Diluire 30 mL della soluzione di lavaggio concentrata con 1170 mL di acqua deionizzata fino ad un volume finale di 1200 mL.
La soluzione di lavaggio diluita è stabile per 2 settimane a temperatura ambiente.

Enzyme Conjugate
Diluire il tracciante enzimatico concentrato 1:10 con il diluente del tracciante.
Stabilità del tracciante enzimatico diluito: 1 settimana a 2 °C - 8 °C in un contenitore chiuso.

Esempio:
Se la piastra intera è usata, diluire 1.2 mL *Enzyme Coniugate* con 10.8 mL del *Conjugate Diluent* per avere un volume totale di 12 mL.
Se non viene usata una piastra intera preparare la quantità del tracciante necessaria mescolando 100 µL del *Enzyme Coniugate 10X* con 0.9 mL del *Conjugate Diluent* per ogni fila di micropozzetti (vedi tabella):

No. di file	*Enzyme Conjugate 10X* conc. (µL)	*Conjugate Diluent* (mL)
1	100	0,9
2	200	1,8
3	300	2,7
4	400	3,6
5	500	4,5
6	600	5,4
7	700	6,3
8	800	7,2
9	900	8,1
10	1000	9,0
11	1100	9,9
12	1200	10,8

4.5 Smaltimento del kit

Lo smaltimento del kit deve avvenire secondo le regole a norma di legge. Informazioni particolareggiate per questo prodotto si trovano nel regolamento di sicurezza, capitolo 13.

4.6 Test kits danneggiati

Nel caso di gravi danneggiamenti del kit o dei suoi componenti deve avvenire una dichiarazione scritta alla ditta DRG, al più tardi una settimana dopo il ricevimento del kit. Componenti danneggiati non dovrebbero essere utilizzati per il test. Questi componenti devono essere magazzinati fino ala soluzione del problema. Dopo di che essi devono essere smaltiti secondo le norme ufficiali.

5 CAMPIONI

Siero o plasma (EDTA-, Eparina- or citrate plasma) può essere usato per questo test.
Non usare campioni emolitici, itterici o lipemici.
Attenzione: Se i campioni contengono sodio azide non devono essere utilizzati per questo test.

5.1 Collezione dei campioni

Siero:
Collezionare sangue tramite puntura venale (p.es. Sarstedt Monovette per siero), far coagulare e separare il siero centrifugando a temperatura ambiente.
Non centrifugare prima che la coagulazione sia completata. Campioni di pazienti con una terapia anticoagulante possono richiedere più tempo per la coagulazione.

Plasma:
Il sangue dovrebbe essere collezionato in tubetti da centrifuga contenenti un anticoagulante (p. es. Sarstedt Monovette con un'adeguata preparazione per il plasma) e centrifugando immediatamente dopo la puntura.

5.2 Magazzinaggio dei campioni

I campioni dovrebbero essere magazzinati ben chiusi fino a 5 giorni a 2 °C - 8 °C.
Campioni magazzinati per un periodo più lungo (fino a 12 mesi) dovrebbero essere congelati a -20 °C prima dell'analisi. Congelare soltanto una volta.
Invertire campioni scongelati alcune volte prima dell'uso.

5.3 Diluizione dei campioni

Se in un campione di siero viene trovata una concentrazione oltre lo standard più alto, questo campione può essere diluito con *Sample Diluent* e nuovamente determinato.
Della diluizione deve essere però tenuto conto.
Esempio:
a) diluizione 1:10: 10 µL campione + 90 µL *Sample Diluent* (agitare bene)
b) diluizione 1:100: 10 µL della diluizione a) + 90 µL *Sample Diluent* (agitare bene).

6 ATTUAZIONE DEL TEST

6.1 Indicazioni generali

- Tutti i reagenti e i campioni devono essere portati a temperatura ambiente e ben mescolati prima dell'uso. Evitare la formazione di schiume.
- Una volta iniziato il procedimento del test, questo deve essere portato alla fine senza interruzione.
- Per ogni componente, standard, controllo o campione è necessario utilizzare una nuova punta monouso per evitare reazioni incrociate.
- La densità ottica dipende dal tempo d'incubazione e dalla temperatura. Perciò si rende necessario di preparare tutti i reagenti, di aprire i tappi dei flaconi e di appostare tutti i pozzetti nelle appropriate posizioni. Soltanto una tale preparazione garantisce gli stessi tempi per ogni processo di pipettamento.
- Come regola generale vale che la reazione enzimatica si svolge linearmente proporzionale con il tempo e con la temperatura.

6.2 Eseguimento del test

Ogni analisi deve includere una curva standard.

1. Fissare i pozzetti necessari sul supporto.
2. Pipettare **20 µL** di ogni *Standard*, *Control* e campione nei pozzetti, cambiando ogni volta la punta monouso.
3. Pipettare **100 µL** *Enzyme Conjugate* (vedi "preparazionedei reagenti") in ogni pozzetto.
   Agitare bene per 10 secondi. È molto importante raggiungere un completo mescolamento.
4. Incubare per **120 minuti** a temperatura ambiente.
5. Rovesciare la piastra per vuotare i pozzetti.
   Lavare i pozzetti 5 volte con *Wash Solution* diluita (400 µL in ogni pozzetto). Rimuovere le gocce d'acqua rimanenti rivoltando la piastra su carta assorbente.
   Importante:
   La sensibilità e la precisazione di questo kit sono fortemente influenzate dal corretto eseguimento del lavaggio!
6. Aggiungere **100 µL** della *Substrate Solution* ad ogni pozzetto.
7. Incubare per **30 minuti** a temperatura ambiente.
8. Fermare la reazione enzimatica aggiungendo **100 µL** della *Stop Solution* ad ogni pozzetto.
9. Determinare la densità ottica a **450** ± **10 nm** con un fotometro per microtiter-piastre **entro 10 minuti** dopo l'aggiunta della *Stop Solution*.

6.3 Rilevamento dei risultati

1. Determinare i valori medi della densità ottica per ogni set di standard, controlli e campioni.
2. Costruire una curva standard: riportare i valori medi della densità ottica (OD) di ogni standard contro la rispettiva concentrazione dove i valori delle OD si devono trovare sull'asse verticale (Y) e le concentrazioni sull'asse orizzontale (X).
3. Utilizzando il valore medio delle OD per ogni campione si determina la rispettiva concentrazione dalla curva standard.
4. Metodo automatico: I valori riportati in questo istruzioni per l'uso sono stati determinati tramite l'equazione a 4 parametri. (I methodi preferiti sono 4 Parameter Rodbard oppure 4 Parameter Marquardt.) Altri funzioni usati per l'elaborazioni dei dati possono dare risultati leggermente differenti.
5. La concentrazione dei campioni può essere determinata direttamente dalla curva standard. Campioni con una concentrazione più elevata dello standard più concentrato devono essere diluiti. Di questo fattore di diluizione deve essere tenuto conto per il calcolo della concentrazione.

6.3.1 Esempio di una curva standard tipica

I seguenti dati sono a scopo dimostrativo soltanto e **non possono** sostituire i dati generati dall'eseguimento del test.

Standard	Densità ottiche (450 nm)
Standard 0 (0 U/mL)	0,05
Standard 1 (3 U/mL)	0,14
Standard 2 (20 U/mL)	0,65
Standard 3 (50 U/mL)	1,25
Standard 4 (100 U/mL)	2,04

7 VALORI NORMALI

È consigliabile che ogni laboratorio determini i propri valori normali e anormali.

In uno studio condotto su persone apparentemente sane usando il test DRG TM-CA 72-4 ELISA i seguenti valori sono stati ottenuti:

Populazione	N Valori	Mediano	Valore medio	5. - 95. Percentile
persone apparentemente sane	65	0.72 U/mL	0.86 U/mL	0 U/mL - 2.68 U/mL

I risultati si accordano con dati di valore soglia publicati (cut-off 4 U/mL - 6 U/mL) (Reference/Literature 3-6).

8 CONTROLLO QUALITÀ

È consigliabile utilizzare i campioni controllo secondo le norme di legge. Attraverso l'utilizzo dei campioni controllo si può raggiungere una verifica dei risultati giorno per giorno. Dovrebbero essere adoperati campioni controllo sia con un livello normale sia con uno patologico.
Le referenze con i rispettivi risultati del laboratorio QC sono elencati nel QC certificato, che è allegato al kit. I valori riportati nel QC certificato si riferiscono al lotto del kit attuale e dovrebbero essere utilizzati per un raffronto dei risultati.
È altresì consigliabile di partecipare a programmi di sicurezza sulla qualità nazionali o internazionali, per assicurarsi dell'esattezza dei risultati.
Appropriati metodi statistici per l'analisi dei valori controllo e delle rappresentazioni grafici dovrebbero essere adoperati. Nel caso che i risultati del test non combaciano con il campo di accettazione indicato dal materiale di controllo, i risultati dei pazienti devono essere considerati invalidi. In questo caso si prega di controllare i seguenti fattori d'errore: pipette, cronometri, fotometro, data di scadenza dei reagenti, condizione di magazzinaggio e d'incubazione, metodi di aspirazione e di lavaggio.
Se dopo il controllo dei suddetti fattori non è rilevabile alcun errore, si prega di contattare il fornitore o direttamente la ditta DRG.

9 CARATTERISTICHE DEL TEST

9.1 Assay Dynamic Range

Le concentrazioni determinabili con questo test stanno tra 0.79 U/mL – 100 U/mL.

9.2 Specificità degli anticorpi (reazioni ad incrocio)

I sieri di individui sani non erano reattivi con il DRG TM-CA 72-4 ELISA

9.3 Sensitività analitica

La sensitività analitica è stata calcolata dai valori medi piu' due deviazioni standard di venti (20) repliche dello *Standard 0* ed erano 0.79 U/mL.

9.4 Precisione

Per dettagli più precisi consultare la metodica in inglese.

9.5 Ritrovato

Per dettagli più precisi consultare la metodica in inglese.

9.6 Linearità

Per dettagli più precisi consultare la metodica in inglese.

10 LIMITAZIONE DEL TEST

Ogni manutenzione impropria dei campioni o modificazione del protocollo può influenzare I risultati.

10.1 Sostanze interferenti

Emoglobina (fino a 4 mg/mL), bilirubina (fino a 0.5 mg/mL) e trigliceridi (fino a 7.5 mg/mL) non influenzano i risultati di questo test.
I risultati trigliceridi > 7.5 mg/mL sono diminuiti.

Il test contiene reagenti in grado di minimizzare l'interferenza di HAMA e di anticorpi eterofilici. Però livelli estremamente alti di HAMA o anticorpi eterofilici possono interferire con i risultati del test.

10.2 Droghe interferenti

Fino ad oggi nessuna sostanza (farmaco) è conosciuta a noi che abbia influenzato la determinazione di CA 72-4 nel campione.

10.3 Effetto Hook di alti dosaggi

Nessun effetto hook (di aglomerazione) è stato osservato in questo test fino a 6400 U/mL di CA 72-4.

11 ASPETTI LEGALI

11.1 Affidabilità dei risultati

Il test deve essere eseguito esattamente secondo il protocollo dato dal produttore. Inoltre l'utente deve seguire le regole del GLP (Good Laboratory Practice) o eventualmente altre regole comportamentali o disposizioni legali. Questo vale soprattutto per l'uso delle referenze. È molto importante utilizzare un numero appropriato di referenze in parallelo ai campioni test per poter controllare l'esattezza e la precisione del test.
I risultati del test sono validi soltanto se tutte le referenze cadono nei margini prestabiliti e se tutti gli altri parametri del test soddisfano la specificazione per questo test. Se esistono dubbi o domande su questi risultati, si prega di contattare la ditta DRG.

11.2 Conseguenze terapeutiche

Soltanto sulla base dei risultati dei laboratori non dovrebbero essere intraprese delle conseguenze terapeutiche di alcun tipo, anche se i risultati del test sono d'accordo con gli aspetti articolati nel punto 11.1. Ogni risultato di laboratorio è soltanto una parte di un quadro clinico completo di un paziente.
Soltanto in casi in cui i risultati di un test del laboratorio si accordano con il quadro clinico dell'ammalato, si possono intraprendere delle conseguenze terapeutiche.
Il risultato del test da solo non è base sufficiente per lo stabilimento di una terapia.

11.3 Responsabilità legali

Ogni cambiamento del protocollo del test e/o lo scambio o il mescolamento di componenti provenienti da cariche diverse possono influenzare negativamente i risultati e compromettere la validità del test. Questi cambiamenti e/o scambi annullano ogni diritto al risarcimento.
Si respingano inoltre tutti i richiami risultanti da interpretazioni sbagliate da parte dell'utente secondo il paragrafo 11.2.
Nel caso di reclamazione, la garanzia del produttore è limitato al valore massimo del test kit. Ogni danno provocato durante il trasporto del kit non sottostà alla responsabilità del produttore.

12 BIBLIOGRAFIA

Per dettagli più precisi consultare la metodica in inglese.

1 INTRODUCCIÓN

El **Kit de inmunoensayo enzimático DRG CA 72-4** proporciona los materiales necesarios para la determinación cuantitativa del CA 72-4 (TAG-72) en suero y plasma
Este ensayo está diseñado solo para diagnóstico *in vitro*.

2 FUNDAMENTO DEL ENSAYO*

El Kit DRG TM-CA 72-4 ELISA es un ensayo en fase sólida de inmunoadsorción unido a enzimas (ELISA), basado en el principio del sándwich.
Los pocillos de las placas están recubiertos con un anticuerpo monoclonal (Clone CC49) dirigido contra un único foci antigénico en una molécula de CA 72-4. Se incuba una alícuota de una muestra perteneciente a un paciente que contiene CA 72-4 endógena en los pocillos recubiertos con el enzima conjugado, que es un anticuerpo anti- CA 72-4 (Clone B72.3) conjugado con la peroxidasa endógena. Después de la incubación se lava el conjugado que no se ha unido.
La cantidad de peroxidasa unida es proporcional a la concentración de CA 72-4 en la muestra.
Cuando se añade la solución del sustrato de la peroxidasa, la intensidad del color desarrollado es proporcional a la concentración de CA 72-4 en la muestra del paciente.

* Los anticuerpos utilizados en este ensayo están protegidos por patentes:

1. U.S. Patent No. 5,512,443, issued April 4, 1996 entitled “Second generation monoclonal antibodies having binding specificity to TAG-72 and human carcinomas and methods for employing the same” (**HHS** Reference No. E-160-1987/0-US-18)
2. Canadian Patent No. 1339980, issued August 4, 1998 entitled “Second generation monoclonal antibodies having binding specificity to TAG-72 and human carcinomas and methods for employing the same” (**HHS** Reference No. E-160-1987/0-CA-04)
3. U.S. Patent No. 4,522,918, issued June 11, 1985 (now expired) entitled “Process for Producing Monoclonal Antibodies Reactive with Human Breast Cancer” (**HHS** Reference No. E-185-1981/0-US-01)

3 PRECAUCIONES

- Este kit es solamente para diagnóstico *in vitro*.
- Por favor, se usa solo la version valida de la metodico técnico incluido aqui en el kit.
- Para obtener información de las sustancias peligrosas incluidas en el kit por favor mirar las hojas de los datos de seguridad del material.
- Todos los reactivos en este kit de ensayo que contienen suero o plasma humano se han ensayado y confirmado ser negativos para HIV I/II, HBsAg y HCV mediante procedimientos aprobados por la FDA. Sin embargo, todos los reactivos deben ser tratados tanto en su uso como dispensación como potencialmente biopeligrosos.
- Evitar contacto con *Stop Solution* que contiene H_2SO_4 0,5 M. Puede provocar irritación y quemaduras en la piel.
- Nunca pipetear con la boca y evitar el contacto de los reactivos y las muestras con la piel y con membranas mucosas.
- No fumar, comer, beber o usar cosméticos en áreas donde las muestras o los reactivos del kit están siendo usados.
- Usar guantes de látex cuando se utilicen las muestras y los reactivos. La contaminación microbiana de los reactivos o las muestras puede dar resultados erróneos.
- El manejo debe realizarse de acuerdo a los procedimientos definidos por las guías o regulación nacionales de seguridad de sustancias biopeligrosas.
- No utilizar los reactivos después de su fecha de caducidad que aparece en las etiquetas del kit.
- Todos los volúmenes indicados han de ser realizados de acuerdo con el protocolo. Los resultados óptimos del ensayo se obtienen solo cuando se utilizan pipetas y lectores de microplacas calibrados.
- No mezclar o usar componentes de kits con distinto número de lote. Se recomienda no intercambiar pocillos de distintas placas incluso si son del mismo lote. Los kits pueden haber sido enviados o almacenados bajo diferentes condiciones y las características de unión de las placas pueden resultar diferentes.
- Los compuestos químicos y los reactivos preparados o utilizados han de tratarse como residuos peligrosos de acuerdo con las guías o regulación nacionales de seguridad de sustancias biopeligrosas.
- Las hojas de los datos de seguridad de este producto están disponibles bajo pedido directamente a DRG Instruments GmbH.

4 COMPONENTES DEL KIT

4.1 Componentes del Kit

1. ***Microtiterwells*** (Placas multipocillo), 12 x 8 tiras separables, 96 pocillos;
   Pocillos recubiertos con anticuerpo anti-CA 72-4 (monoclonal).
2. ***Standard (Standard 0-4)*****,** (Estándar)**,** 5 viales, 0,5 mL, listo para usar;
   Concentraciones: 0; 3, 20, 50, 100 U/mL
   contiene conservante sin mercurio.
3. ***Control Low & High***, (control) 2 viales (liofilizado), 0,5 mL,
   ver “Preparación de los Reactivos”
   Referir los valores y rangos del control a la etiqueta del vial o a la Hoja de datos QC.
   Contiene conservante sin mercurio.
4. ***Sample Diluent*** (Solución para dilución de la muestra), 1 vial, 3,0 mL, listo para usar,
   Contiene conservante sin mercurio.
5. ***Enzyme Conjugate 10X concentrate*** (Conjugado enzimático, 10X), 1 vial, 1,4 mL,
   Anticuerpo anti-CA 72-4 conjugado con la Peroxidasa de rábano;
   ver “Preparación de los Reactivos”
   Contiene conservante sin mercurio.
6. ***Conjugate Diluent*** (Solución para dilución del conjugado), 1 vial, 14 mL, listo para usar
   Contiene conservante sin mercurio.
7. ***Substrate Solution*** (Solución de sustrato), 1 vial, 14 mL, listo para usar,
   Tetrametilbencidina (TMB).
8. ***Stop Solution*** (Solución de parada), 1 vial, 14 mL, listo para usar,
   contiene 0.5M H_2SO_4,
   Evitar el contacto con la Solución de parada. Puede causar irritación y quemaduras en al piel.
9. ***Wash Solution*** (Solución de lavado), 1 vial, 30 mL (concentrado 40X),
   ver “Preparación de los Reactivos”.

Note: Se puede solicitar el *Sample Diluent* para la dilución de la muestra.

4.2 Equipamiento y material requerido pero no provisto

- Lector de microplacas calibrado (450 ± 10 nm) (ej. DRG Instruments Microtiter Plate Reader).
- Micropipetas de precisión variable calibradas.
- Papel absorbente.
- Agua destilada.

4.3 Almacenamiento y estabilidad del kit

Cuando se almacena a 2 °C - 8 °C, los reactivos sin abrir mantienen su reactividad hasta la fecha de caducidad. No utilizar los reactivos más allá de esta fecha.
Los reactivos abiertos han de almacenarse a 2 °C - 8 °C. Las placas multipocillo han de almacenarse a 2 °C - 8 °C.
Una vez se ha abierto la bolsa hay que tener cuidado y cerrarla de nuevo.
Los kits abiertos conservan su actividad durante dos meses si se almacenan como se ha descrito arriba.

4.4 Preparación de los Reactivos

Dejar que todos los reactivos y el número requerido de tiras alcancen la temperatura ambiente antes de usarse.

Control
Reconstituir el contenido liofilizado con 0,5 mL de agua destilada y dejar reposar como mínimo durante 10 minutos. Mezclar los controles varias veces antes de usar.
Nota: *Los controles reconstituidos debe alicuotarse y almacenarse a -20 °C.*

Wash Solution
Mezclar 30 mL de la solución de lavado concentrada con 1170 mL de agua desionizada hasta un volumen final de 1200 mL.
La solución del lavado diluida es estable durante 2 semanas a temperatura ambiente.

Enzyme Conjugate
Diluir el *Enzyme Conjugate* concentrado 1:10 en el *Conjugate Diluent*.
Estabilidad del Conjugado-Enzimático preparado: 1 semana a 2 °C - 8 °C en un recipiente cerrado.

Ejemplo:
Si se utiliza toda la placa, mezclar 1,2 mL del *Enzyme Conjugate* con 10.8 mL *Conjugate Diluent* hasta un volumen final de 12 mL.
Si no se utiliza toda la placa de una vez, preparar la cantidad de Conjugado-Enzimático requerido mediante la mezcla de 100 µL del *Enzyme Conjugate 10X* conc. con 0.9 mL del *Conjugate Diluent* por tira (ver tabla mas abajo):

No. of strips	*Enzyme Conjugate 10X conc*. (µL)	*Conjugate Diluent* (mL)
1	100	0,9
2	200	1,8
3	300	2,7
4	400	3,6
5	500	4,5
6	600	5,4
7	700	6,3
8	800	7,2
9	900	8,1
10	1000	9,0
11	1100	9,9
12	1200	10,8

4.5 Eliminación del Kit

La eliminación del kit debe realizarse de acuerdo con las leyes nacionales. En las hojas de datos de seguridad se proporciona información especial de este producto (ver capítulo 13).

4.6 Kits de ensayo dañados

En caso de que exista cualquier daño severo del kit de ensayo o de sus componentes, ha de informarse por escrito a DRG, no mas tarde de una semana después de recibir el kit. No deben utilizarse componentes dañados para llevar a cabo un ensayo. Han de almacenarse hasta que se encuentre una solución. Después de esto, deben ser eliminados de acuerdo con las leyes oficiales.

5 MUESTRAS

En este ensayo pueden usarse suero o plasma (plasma EDTA, Heparina o citrato).
No usar muestras hemolíticas, ictéricas o lipémicas.
Tener en cuenta: No deben usarse muestras que contengan acida sódica.

5.1 Toma de muestras

Suero:
Recoger la sangre por punción en la vena (ej. Sarstedt Monovette para el suero), permitir coagulación, y separar el suero por centrifugación a temperatura ambiente. No centrifugar antes de la coagulación completa. Las muestras de pacientes que reciben terapia anticoagulante requieren más tiempo para coagular.

Plasma:
Toda la sangre ha de recogerse en tubos de centrífuga que contengan anticoagulante (Ej. Sarstedt Monovette con una preparación adecuada para el plasma) y centrifugar inmediatamente tras la recogida.

5.2 Almacenamiento de las muestras

Las muestras deben ser tapadas y pueden ser almacenadas hasta 5 días a 2 °C - 8 °C antes del ensayo.
Las muestras almacenadas por un período de tiempo mas largo (hasta 12 meses) han de congelarse vez a -20 °C antes del ensayo. Las muestras descongeladas deben invertirse varias veces antes del ensayo.

5.3 Dilución de las muestras

Si en un ensayo inicial, se encuentra una muestra que presenta valores mayores que el estándar mas concentrado, ha de diluirse con *Sample Diluent* y volver a ensayarse como se describe en el Procedimiento de Ensayo.
Para el cálculo de las concentraciones habrá que tener en cuenta el factor de dilución.
Ejemplo:
a) dilución 1:10: 10 µL muestra + 90 µL *Sample Diluent* (mezclar totalmente)
b) dilución 1:100: 10 µL dilución a) 1:10 + 90 µL *Sample Diluent* (mezclar totalmente).

6 PROCEDIMIENTO DE ENSAYO

6.1 Consideraciones generales

- Todos los reactivos y muestras han de estar a temperatura ambiente antes de su uso. Todos los reactivos deben mezclarse sin formar espuma.
- Una vez se ha comenzado el ensayo deben completarse todos los pasos sin interrupción.
- Utilizar puntas de pipeta de plástico nuevas para cada estándar, control o muestra para evitar combinaciones cruzadas.
- La absorbancia es función del tiempo de incubación y la temperatura. Antes de comenzar el ensayo, se recomienda que todos los reactivos estén preparados, tapas removidas, todos los pocillos que se necesiten asegurados en recipiente, etc. Esto asegurará un tiempo similar para cada paso de pipeteo sin que haya interrupciones.
- Como regla general, la reacción enzimática es linealmente proporcional al tiempo y a la temperatura.

6.2 Procedimiento de ensayo

Cada uno debe incluir una curva de estándares.

1. Asegurar el número deseado de pocillos en el recipiente.
2. Dispensar **20 µL** de cada *Standard, Control* y muestras con puntas nuevas en los pocillos adecuados.
3. Dispensar **100 µL** de *Enzyme Conjugate* (ver "Preparación de los Reactivos") a cada pocillo.
4. Mezclar totalmente durante 10 segundos. Es importante mezclar completamente en este paso.
5. Incubar durante **120 minutes** a temperatura ambiente.
6. Sacudir enérgicamente el contenido de los pocillos.
   Lavar los pocillos 5 veces con *Wash Solution* diluida (400 µL por pocillo). Realizar un golpe seco de los pocillos contra el papel absorbente para eliminar las gotas residuales.
   Nota importante:
   La sensibilidad y la precisión de este ensayo se ve marcadamente influenciada por la realización correcta del proceso de lavado!
7. Adicionar **100 µL** de *Substrate Solution* a cada pocillo.
8. Incubar durante **30 minutes** a temperatura ambiente.
9. Parar la reacción enzimática mediante la adición de **100 µL** de *Stop Solution* a cada pocillo.
10. Leer la OD a **450 ± 10 nm** con un lector de microplacas **dentro de los 10 minutos** después de la adición de la *Stop Solution*.

6.3 Cálculo de los Resultados

1. Calcular los valores de absorbancia media para cada conjunto de estándares, controles y muestras de pacientes.
2. Construir una curva estándar mediante la representación de la absorbancia media obtenida para cada estándar frente a su concentración con el valor de absorbancia en el eje vertical (Y) y la concentración en el eje horizontal (X).
3. Usando el valor de absorbancia media de cada muestra determinar la concentración correspondiente a partir de la curva estándar.
4. Método automatizado: Los resultados en las instrucciones de uso se han calculado automáticamente usando una curva de regresión 4 Parámetros. (4 Parámetros Rodbard o 4 Parámetros Marquardt son los métodos preferidos.) Otras funciones de regresión darán lugar a resultados sensiblemente diferentes.
5. La concentración de las muestra puede leerse directamente de la curva de estándares. Las muestras con concentraciones superiores al mayor estándar han de diluirse. Para el cálculo de las concentraciones hay que tener en cuenta el factor de dilución.

6.3.1 Ejemplo de una Curva Estándar Típica

Los siguientes datos son solamente para la explicación y **no** pueden ser utilizados en lugar de los datos generados en el momento del ensayo.

Estándar	Unidades Ópticas (450 nm)
Standard 0 (0 U/mL)	0,05
Standard 1 (3 U/mL)	0,14
Standard 2 (20 U/mL)	0,65
Standard 3 (50 U/mL)	1,25
Standard 4 (100 U/mL)	2,04

7 VALORES ESPERADOS

Se recomienda encarecidamente que cada laboratorio determine sus valores normales e inusuales.

En un estudio con adultos aparentemente sanos utilizando el DRG TM-CA 72-4 ELISA se observaron los siguientes valores:

Población	N válido	Mediana	Media	Percentil 5. - 95.
adultos aparentemente sanos	65	0.72 U/mL	0.86 U/mL	0 U/mL - 2.68 U/mL

Los resultados corresponden bien con valores del cut-off publicados entre 4 U/mL - 6 U/mL (Reference/Literature 3-6).

8 CONTROL DE CALIDAD

Se recomienda usar muestras control de acuerdo con las leyes estatales y federales. El uso de muestras control ser recomienda para asegurar la validez diaria de los resultados. Usar controles tanto a niveles normal como patológico. Los controles y los correspondientes resultados del Laboratorio de control de calidad están fijados en el certificado de control de calidad que acompañan al kit. Los valores y los rangos fijados en la hoja del control de calidad se refieren siempre al kit actual y deben usarse para la comparación directa de los resultados.
Es recomendable también hacer uso de programas de Aseguramiento de la Calidad nacionales o internacionales para asegurar la exactitud de los resultados.
Utilizar métodos estadísticos apropiados para el análisis de los valores y tendencia de los controles. Si los resultados del ensayo no se ajustan a los rangos aceptables establecidos en los controles, los resultados obtenidos de los pacientes han de considerarse inválidos.
En este caso, por favor comprobar las siguientes áreas técnicas: Pipeteo y tiempo empleado, fotómetro, fecha de caducidad de los reactivos, condiciones de almacenamiento e incubación, métodos de aspiración y lavado.
Después de comprobar los asuntos mencionados arriba sin encontrar ningún error, contactar con su distribuidor o con DRG directamente.

9 CARACTERÍSTICAS DEL ENSAYO

9.1 Rango dinámico del ensayo

El rango del ensayo se encuentra entre 0,79 U/mL – 100 U/mL.

9.2 Especificidad de los Anticuerpos (Reactividad Cruzada)

Consultar el manual de usuario en inglés.

9.3 Sensibilidad Analítica

La sensibilidad analítica se calculó a partir de la media mas dos desviaciones estándar de veinte (20) réplicas del *Estándar 0* y resultó ser 0,79 U/mL.

9.4 Precisión

Consultar el manual de usuario en inglés.

9.5 Recuperación

Consultar el manual de usuario en inglés.

9.6 Linealidad

Consultar el manual de usuario en inglés.

10 LIMITACIONES DE USO

Cualquier manipulación inadecuada de las muestras o modificaciones del ensayo pueden influenciar los resultados.

10.1 Sustancias que pueden interferir

Hemoglobina (hasta 4 mg/mL), Bilirrubina (hasta 0.5 mg/mL) y Triglicéridos (hasta 7,5 mg/mL) no influencian los resultados del ensayo.
Triglicéridos > 7.5 mg/mL sufre disminución de sus valores.

El ensayo contiene reactivos para minimizar la interferencia de HAMA y de anticuerpos heterofílicos. Sin embargo, títulos extremadamente elevados de HAMA o anticuerpos heterofílicos pueden interferir con los resultados del ensayo.

10.2 Interferencias con drogas

Hasta ahora no se han encontrado sustancias (drogas) conocidas por nosotros, que tengan influencia en la medida de CA 72-4 en una muestra.

10.3 Efecto Gancho-Dosis-Elevada

No se ha observado efecto gancho en este ensayo hasta 6400 U/mL de CA 72-4.

11 ASPECTOS LEGALES

11.1 Fiabilidad de los Resultados

El ensayo debe realizarse exactamente de acuerdo a las instrucciones del fabricante. Mas aún, el usuario debe ajustarse estrictamente a las reglas BPL (Buenas Prácticas de Laboratorio) o a otros estándares y/o leyes nacionales aplicables. Esto es especialmente relevante para el uso de reactivos control. Es importante incluir siempre, dentro del procedimiento de ensayo, un número suficiente de controles para validar la exactitud y la precisión del ensayo.
Los resultados del ensayo son válidos sólo si todos los controles se encuentran dentro de los rangos especificados y si todos los otros parámetros del ensayo se encuentran dentro de las especificaciones dadas para el ensayo. En cado de alguna duda o inquietud, por favor, contactar con DRG.

11.2 Consecuencias Terapéuticas

Las consecuencias terapéuticas nunca deben basarse sólo en los resultados de laboratorio incluso si todos los resultados del ensayo están de acuerdo con los asuntos fijados en el punto 11.1. Cualquier resultado de laboratorio es solamente una parte del cuadro clínico de un paciente.
Solamente en los casos donde los resultados de laboratorio están en acuerdo con todo el cuadro clínico de un paciente, se pueden derivar consecuencias terapéuticas.
Nunca deben derivarse consecuencias terapéuticas a partir de solamente el resultado obtenido en el ensayo

11.3 Responsabilidad

Cualquier modificación del kit y/o cambio o mezcla de cualquier componente procedentes de kits de lotes diferentes puede afectar negativamente a los resultados esperados y en la validez de todo el test. Esas modificaciones y/o cambios invalidad cualquier reclamación de reposición.
Las reclamaciones emitidas debidas a una mala interpretación de los resultados de laboratorio por parte del comprador referidos al punto 11.2 son también inválidas. A pesar de todo, en el caso de cualquier reclamación, la responsabilidad del fabricante no excede el valor del kit. Cualquier daño provocado al kit durante su transporte no está sujeto a la responsabilidad del fabricante.

12 REFERENCIAS / BIBLIOGRAFÍA

Consultar el manual de usuario en inglés.

1 INTRODUCTION

Le kit de dosage immuno-enzymatique **DRG TM-CA 72-4 ELISA** propose les matériaux requis pour la mesure quantitative de CA 72-4 (TAG-72) dans le sérum ou le plasma.
Ce kit est à utiliser uniquement dans le cadre de tests diagnostiques in vitro.

2 PRINCIPE DU TEST*

Le kit DRG TM-CA 72-4 ELISA est basé sur une réaction immuno-enzymatique en sandwich en phase solide.
Les microplaques sont recouvertes avec un anticorps monoclonal (Clone CC49) dirigé contre un antigène spécifique de la molécule CA 72-4. Un aliquot de l'échantillon contenant le (la) CA 72-4 endogène est incubé dans un puits avec l'enzyme conjuguée, c'est-à-dire un anticorps anti- CA 72-4 (Clone B72.3) conjuguée avec la peroxidase de Raifort (horseradish peroxidase, HRP). Apres l'incubation, le conjugué non-lié est éliminé durant le lavage des puits. La quantité de conjugué-HRP liée est proportionnelle à la concentration de CA 72-4 contenu(e) dans l'échantillon. Suite à l'addition de solution substrat, l'intensité de la coloration obtenue est proportionnelle à la concentration de CA 72-4 contenu(e) dans l'échantillon.

* Les anticorps utilisés dans ce test sont protégés par des brevets:

1. U.S. Patent No. 5,512,443, issued April 4, 1996 entitled "Second generation monoclonal antibodies having binding specificity to TAG-72 and human carcinomas and methods for employing the same" (**HHS** Reference No. E-160-1987/0-US-18)
2. Canadian Patent No. 1339980, issued August 4, 1998 entitled "Second generation monoclonal antibodies having binding specificity to TAG-72 and human carcinomas and methods for employing the same" (**HHS** Reference No. E-160-1987/0-CA-04)
3. U.S. Patent No. 4,522,918, issued June 11, 1985 (now expired) entitled "Process for Producing Monoclonal Antibodies Reactive with Human Breast Cancer" (**HHS** Reference No. E-185-1981/0-US-01)

3 PRECAUTIONS D'UTILISATION

- Ce kit est uniquement destiné aux tests diagnostiques in vitro.
- Les informations concernant la toxicité des réactifs contenus dans ce kit sont présentées dans la fiche de sécurité (« Material Safety Data Sheets »).
- Tous les réactifs de ce kit contenant du sérum ou du plasma humain ont été testés avec des résultats négatifs pour le VIH I/II, le HBsAg et le HCV selon les normes FDA en vigueur. Néanmoins, lors de leur utilisation, tous les réactifs de ce kit doivent être manipulés avec précaution.
- Eviter les contacts avec la *Stop Solution*, celle-ci contient 0.5 M de H_2SO_4. Cela pourrait engendrer irritations ou brûlures de la peau.
- Ne jamais pipeter avec la bouche, et éviter tout contact de la peau ou des muqueuses avec les réactifs ou les échantillons.
- Ne pas fumer, manger, boire ou utiliser des produits cosmétiques dans les zones où les échantillons ou le kit ont été maniés.
- Porter des gants d'examen lors de l'utilisation des échantillons ou des réactifs. Une contamination microbienne des échantillons ou des réactifs pourrait fausser les résultats.
- L'utilisation de ce kit devra être en accord avec les normes ou recommandations nationales de sécurité en vigueur concernant les produits à risque biologique.
- Ne pas utiliser les réactifs au-delà de la date d'expiration inscrite sur l'emballage.
- Tous les volumes indiqués doivent être scrupuleusement respectés, comme indiqué dans le protocole expérimental. Seule l'utilisation de pipettes calibrées ou d'un spectrophotomètre lecteur de micro-plaques calibré garantit l'obtention de résultats optimaux à ce test.
- Ne pas mélanger ou utiliser des réactifs contenus dans des kits de lots différents. Il est conseillé de ne pas échanger les puits de différentes plaques, même si celles-ci proviennent du même lot. Les kits peuvent avoir été transportés ou stockés différemment, et les caractéristiques de liaison de chaque plaque pourraient ainsi être modifiées.
- L'élimination des solutions chimiques et des réactifs contenus dans ce kit, utilisés ou non, doit être en accord avec la réglementation nationale en vigueur concernant l'élimination des déchets à risque biologique.
- La fiche de sécurité concernant ce produit peut être obtenue en contactant directement DRG Instruments GmbH.

4 COMPOSITION DU KIT

4.1 Contenu du kit

1. ***Microtiterwells*** *(Plaques de micro-titratio)*, 12 x 8 (à détacher) barrettes, plaques de 96 puits;
   Les puits sont recouverts avec un anticorps anti-CA 72-4 (monoclonal).
2. ***Standard (Standard 0-4)*****,** 5 flacons, 0,5 mL, prêt à l'emploi ;
   Concentrations: 0; 3, 20, 50, 100 U/mL.
   contient agent de conservation sans mercure.
3. ***Control Low & High*** *(Contrôle)*, 2 flacons (lyophilisé), 0,5 mL,
   voir « Préparation des réactifs »
   Les valeurs contrôles et limites sont indiquées sur l'étiquette du flacon ou sur la fiche QC.
   Contient agent de conservation sans mercure.
4. ***Sample Diluent*** *(Solution pour dilution de l'échantillon)*, 1 flacon, 3,0 mL, prêt à l'emploi,
   contient agent de conservation sans mercure.
5. ***Enzyme Conjugate 10X concentrate*** *(Conjugué enzymatique)*, 1 flacon, 1.4 mL,
   anticorps anti-CA 72-4 conjugué à la HRP;
   voir « Préparation des réactifs ».
   Contient agent de conservation sans mercure.
6. ***Conjugate Diluent*** *(Solution pour dilution du conjugué)*, 1 flacon, 14 mL, prêt à l'emploi.
   Contient agent de conservation sans mercure.
7. ***Substrate Solution*** *(Solution substrat)*, 1 flacon, 14 mL, prêt à l'emploi,
   Tétraméthylbenzidine (TMB).
8. ***Stop Solution*** *(Solution d'arrêt)*, 1 flacon, 14 mL, prêt à l'emploi,
   contient 0.5M de H_2SO_4,
   Eviter les contacts avec la solution stop. Cela pourrait engendrer irritations ou brûlures de la peau.
9. ***Wash Solution*** *(Solution de lavage)*, 1 flacon (concentré 40X),
   voir « Préparation des réactifs ».

Remarque : Un *Sample Diluent* pour la dilution de l'échantillon peut être fourni sur demande.

4.2 Equipement et matériel requis, mais non fournis

- Un spectrophotomètre lecteur de micro-plaques calibré (450 ± 10 nm) (ex. le lecteur de microplaques de DRG Instruments GmbH).
- Des micro-pipettes de précision variables et calibrées.
- Du papier absorbant.
- De l'eau distillée.

4.3 Stockage et stabilité du kit

Les réactifs contenus dans des flacons non-ouverts, stockés à 2 °C - 8 °C, seront stables jusqu'à la date d'expiration inscrite sur l'étiquette. Ne pas utiliser les réactifs au-delà de cette date.
Les réactifs contenus dans des flacons ouverts doivent être stockés à 2 °C - 8 °C. Les micro-plaques doivent être stockées à 2 °C - 8 °C. Une fois la capsule d'aluminium ouverte, attention à bien refermer le flacon.
Les kits ouverts conservent leur activité durant deux mois s'ils sont stockés comme précédemment mentionné.

4.4 Préparation des réactifs

Amener tous les réactifs et le nombre de barrettes nécessaires au test à température ambiante avant utilisation.

Control
Reconstituer le contenu lyophilisé avec 0,5 mL d'eau distillée et laisser incuber au minimum 10 minutes. Mélanger les contrôles plusieurs fois avant utilisation.
Remarque : *Les contrôles reconstitué doit être aliquoté et stocké à -20 °C.*

Wash Solution
Diluer 30 mL de solution de lavage concentrée avec 1170 mL d'eau désionisée, pour un volume final de 1200 mL.
Remarque : *La solution de lavage diluée est stable deux semaines à température ambiante.*

Enzyme Conjugate
Diluer l'*Enzyme Conjugate* concentrée à 1:10 dans le *Conjugate Diluent*.
Remarque : *Stabilité de l'enzyme conjuguée diluée : une semaine à 2 °C - 8 °C dans un container scellé.*

Exemple:
Si la plaque entière est utilisée, diluer 1.2 mL d'*Enzyme Conjugate* avec 10.8 mL *Conjugate Diluent*, pour un volume final de 12 mL.
Si la plaque entière n'est pas utilisée en une seule fois, préparer la quantité nécessaire d'enzyme conjuguée en mélangeant 100 µL d'*Enzyme Conjugate 10X* conc. avec 0.9 mL de *Conjugate Diluent* par barrette (voir la table ci-dessous):

No. of strips	*Enzyme Conjugate 10X conc*. (µL)	*Conjugate Diluent* (mL)
1	100	0,9
2	200	1,8
3	300	2,7
4	400	3,6
5	500	4,5
6	600	5,4
7	700	6,3
8	800	7,2
9	900	8,1
10	1000	9,0
11	1100	9,9
12	1200	10,8

4.5 Elimination des déchets relatifs au kit

L'élimination des déchets relatifs au kit doit être réalisée selon les règles nationales en vigueur. Les informations spécifiques au kit sont présentées dans la fiche de sécurité (voir chapitre 13).

4.6 Kits endommagés

Dans le cas de dommages importants survenus au kit ou ses composants, informer la DRG, au plus tard une semaine après réception du kit. Les composants endommagés ne doivent pas être utilisés pour le test. Ils doivent être stockés jusqu'à ce qu'une solution adaptée ait été trouvée. Après cela, ils doivent être éliminés selon les directives officielles en vigueur.

5 ECHANTILLON

Sérum ou plasma (EDTA-, Héparine- ou citrate plasma) peuvent être utilisés pour ce test.
Ne pas utiliser des échantillons hémolysés, ictériques ou lipémiques.
Remarque : Les échantillons contenant de l'azide de sodium ne doivent pas être utilisés pour ce test.

5.1 Prélèvement et préparation des échantillons

Sérum:
Prélever le sang par ponction veineuse (ex. Sarstedt Monovette pour sérum), laisser coaguler, puis séparer le sérum par centrifugation à température ambiante. Ne pas centrifuger avant que la coagulation ne soit terminée. Les patients sous traitement anti-coagulant peuvent demander un temps de coagulation plus important.

Plasma:
Le sang total doit être prélevé dans des tubes de centrifugation contenant un anti-coagulant (Sarstedt Monovette avec une préparation appropriée de plasma) et centrifugé immédiatement après le prélèvement.

5.2 Conservation des échantillons

Les tubes contenant les échantillons doivent être fermés et peuvent être stockés jusqu'à cinq jours à 2 °C - 8 °C avant d'être testés.
Les échantillons stockés pour un temps prolongé (jusqu'à 12 mois) doivent être congelés à -20 °C avant d'être testés.
Les échantillons décongelés doivent être retournés plusieurs fois avant le test.

5.3 Dilution de l'échantillon

Si, lors d'un test préliminaire, la concentration de l'échantillon se révèle être supérieure à celle du standard le plus concentré, alors l'échantillon doit être dilué avec le *Sample Diluent* et testé de nouveau, comme décrit dans Réalisation du test.
Pour le calcul des concentrations, ce facteur de dilution doit être pris en considération.
Exemple:
a) dilution 1:10: 10 µL l'échantillon + 90 µL *Sample Diluent* (bien mélanger).
b) dilution 1:100: 10 µL dilution a) 1:10 + 90 µL *Sample Diluent* (bien mélanger).

6 RÉALISATION DU TEST

6.1 Remarques générales

- Tous les réactifs et échantillons doivent être amenés à température ambiante avant utilisation. Tous les réactifs doivent être mélangés, sans formation de mousse.
- Une fois la procédure engagée, toutes les étapes doivent être réalisées sans interruption.
- Utiliser un nouveau cône de pipette pour chaque standard, contrôle ou échantillon, ceci afin d'éviter toute contamination.
- L'absorbance est fonction du temps d'incubation et de la température. Avant de commencer le test, il est recommandé de préparer tous les réactifs, bouchons ouverts, de préparer les puits des microplaques, etc. Cela garantira un intervalle de temps équivalent entre chaque étape, sans interruption.
- En règle générale, la réaction enzymatique est linéairement proportionnelle au temps et à la température.

6.2 Réalisation du dosage

Chaque test doit inclure une courbe étalon.

1. Disposer le nombre de puits de micro-titration désiré dans le support.
2. Déposer **20 µL** de chaque *Standard, Control* et les échantillons, avec de nouveaux cônes de pipette, dans les puits appropriés.
3. Déposer **100 µL** d'*Enzyme Conjugate* (voir « Préparation des réactifs ») dans chaque puits.
   Bien mélanger pendant 10 secondes. Il est important d'obtenir un mélange parfait lors de cette étape.
4. Incuber pendant **120 minutes** à température ambiante.
5. Décanter le contenu des puits et rincer les puits 5 fois avec de la *Wash Solution* diluée (400 µL par puits).
   Tapoter les puits sur du papier absorbant afin d'éliminer les gouttelettes résiduelles.
   Remarque importante:
   La sensibilité et la précision de ce test sont fortement dépendantes de la bonne réalisation des étapes de lavage!
6. Ajouter **100 µL** de *Substrate Solution* à chaque puits.
7. Incuber pendant **30 minutes** à température ambiante.
8. Stopper la réaction enzymatique en ajoutant **100 µL** de *Stop Solution* à chaque puits.
9. Lire la densité optique à **450 ± 10 nm** à l'aide d'un spectrophotomètre lecteur de micro-plaques **dans les 10 minutes** après avoir ajouté la *Stop Solution*.

6.3 Calcul des résultats

1. Calculer les valeurs moyennes des densités optiques pour chaque série de standards, contrôles et échantillons.
2. Etablir la courbe étalon en reportant la densité optique moyenne de chaque valeur standard en fonction de sa concentration, en posant la densité optique en axe des ordonnées et la concentration en axe des abscisses.
3. L'utilisation de la densité optique moyenne pour chaque échantillon détermine la concentration correspondante à partir de la courbe étalon.
4. Méthode automatique. Les résultats dans les instructions d'utilisation ont été calculés de façon automatique en utilisant une courbe de régression 4 Paramètres. (4 paramètres Rodbard ou 4 paramètres Marquardt sont les méthodes favorites.) D'autres fonctions logistiques peuvent donner des résultats légèrement différents.
5. La concentration des échantillons peut être lue directement à partir de cette courbe étalon. Les échantillons avec une concentration supérieure à celle du plus haut standard doivent être dilués de nouveau. Pour le calcul des concentrations, ce facteur de dilution doit être pris en considération.

6.3.1 Exemple d'une courbe standard typique

Les résultats suivants sont ici présentés à titre d'exemple et **ne peuvent** être utilisés au moment de l'essai.

Standard	Unités optiques (450 nm)
Standard 0 (0 U/mL)	0,05
Standard 1 (3 U/mL)	0,14
Standard 2 (20 U/mL)	0,65
Standard 3 (50 U/mL)	1,25
Standard 4 (100 U/mL)	2,04

7 VALEURS ATTENDUES

Il est fortement recommandé à chaque laboratoire de déterminer ses propres valeurs normales ou anormales.

Dans une étude réalisée avec des adultes normaux et sains, à l'aide du kit DRG TM-CA 72-4 ELISA, les valeurs suivantes sont observées :

Population	Valid N	Médiane	Valeur moyenne	Percentile 5. - 95.
adultes normaux et sains	65	0.72 U/mL	0.86 U/mL	0 U/mL - 2.68 U/mL

Les résultats correspondent bien avec les valeurs (cut-off) publiés entre 4 U/mL - 6 U/mL (Reference/Literature 3-6).

8 CONTROLE DE QUALITE

Il est recommandé d'utiliser les échantillons contrôles selon les réglementations nationales en vigueur. L'utilisation des échantillons contrôles est recommandé afin de s'assurer jour après jour de la validité des résultats. Utiliser les contrôles de valeurs normales et pathologiques.
Les contrôles et les résultats correspondants issus du laboratoire QC sont mentionnés dans le certificat QC fourni avec le kit. Les valeurs et les limites mentionnées sur la fiche QC font toujours référence au lot de kit courant et doivent être utilisées pour une comparaison directe avec les résultats.
Il est également recommandé d'utiliser les programmes d'évaluation de qualité nationaux ou internationaux, afin de s'assurer de l'exactitude des résultats.
Utiliser les méthodes d'analyses statistiques appropriées pour l'analyse des valeurs contrôles et des tendances. Si les résultats ne correspondent pas aux limites établies des contrôles, les résultats concernant ces patients doivent être considérées comme non valides.
Dans ce cas, tester les zones techniques suivantes : mécanisme de pipettage et temps; spectrophotomètre, dates d'expiration des réactifs, conditions de stockage et d'incubation, méthodes d'aspiration et de lavage.
Après avoir tester les points mentionnés, si aucune erreur n'est détectée, contacter votre distributeur ou directement la DRG.

9 CARACTERISTIQUES DU TEST

9.1 Zone de mesure

Les limites du dosage sont comprises entre 0,79 U/mL – 100 U/mL.

9.2 Spécificité des anticorps (Réaction croisée)

Voir le manuel d'utilisateur en version anglaise.

9.3 Sensibilité de l'analyse

La sensibilité de l'analyse a été calculée à partir de la moyenne la plus élevée de deux déviations standards de l'analyse de vingt réplicats du *Standard 0* et a été mesurée à 0,79 U/mL.

9.4 Précision

Voir le manuel de l'utilisateur en version anglaise.

9.5 Recouvrement

Voir le manuel de l'utilisateur en version anglaise.

9.6 Linéarité

Voir le manuel de l'utilisateur en version anglaise.

10 LIMITES D'UTILISATION

Toute utilisation impropre des échantillons ou toute modification du test peut influencer les résultats.

10.1 Substances parasites

L'hémoglobine (jusqu'à 4 mg/mL), la bilirubine (jusqu'à 0.5 mg/mL) et les triglycérides (jusqu'à 7,5 mg/mL) n'ont aucune influence sur les résultats du dosage.
Triglycérides > 7.5 mg/mL engendre une diminution des valeurs,

Ce test contient des réactifs permettant de minimiser les interférences de l'HAMA ou des anticorps hétérophiliques. Néanmoins, des titres très élevés en HAMA ou en anticorps hétérophiliques peuvent interférer avec les résultats du test.

10.2 Drogues parasites

Jusqu'à présent, nous ne connaissons aucune substance (drogues) capable d'influencer la mesure de CA 72-4 dans un échantillon.

10.3 Effet de surdosage

Jusqu' à 6400 U/mL de CA 72-4, aucun effet de surdosage n'a été détecté avec ce test.

11 ASPECTS LEGAUX

11.1 Fiabilité des résultats

Ce test doit être exactement utilisé selon les instructions d'utilisation du fabricant. De plus, les utilisateurs doivent strictement respecter les règles de la bonne pratique de laboratoire, ou autres lois nationales. Cela est spécialement le cas pour l'utilisation des réactifs contrôles. Pour chaque test, il est important d'inclure un nombre suffisant de contrôles, afin de pouvoir valider l'exactitude et la précision du test.
Les résultats du test sont valides si et seulement si tous les contrôles sont compris dans les gammes de mesure mentionnées et si tous les autres paramètres du test sont également compris dans les instructions de ce test. En cas de doute ou d'inquiétude, contacter la DRG.

11.2 Conséquences thérapeutiques

Les suites thérapeutiques ne devront jamais être basées sur les résultats de laboratoire seuls, même si les tous les résultats du test sont en accord avec les points mentionnés dans le paragraphe 11.1. Tout résultat n'est qu'une partie du tableau clinique complet d'un patient.
Les suites thérapeutiques peuvent découler des résultats de laboratoire si et seulement si ceux-ci sont en accord avec l'ensemble du tableau clinique du patient.
Le résultat du test en lui-même ne doit en aucun cas être le seul déterminant des suites thérapeutiques à suivre.

11.3 Responsabilité

Toute modification du kit et / ou échange ou mélange d'un des composants de différents lots, d'un kit à un autre, pourrait affecter de façon négative les résultats attendus et la validité du test dans son ensemble. De telles modifications ou échanges invalident toute réclamation pour remplacement.
Toutes les réclamations soumises, relatives au paragraphe 11.2, et dues à une mauvaise interprétation des résultats de laboratoire de la part du client sont également invalides. Néanmoins, en cas de réclamation, la responsabilité du fabricant n'est pas de dépasser les limites de la valeur du kit. Tout dommage causé au kit lors de son transport n'est pas du ressort de la responsabilité du fabricant.

12 REFERENCES / BIBLIOGRAPHIE

Voir le manuel de l'utilisateur en version anglaise.

SYMBOLS USED WITH DRG ELISAS

Symbol	English	Deutsch	Français	Español	Italiano
CE	European Conformity	CE-Konformitäts-kennzeichnung	Conforme aux normes européennes	Conformidad europea	Conformità europea
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las Instrucciones	Consultare le istruzioni per l'uso
IVD	In vitro diagnostic device	In-vitro-Diagnostikum	Usage Diagnostic in vitro	Para uso Diagnóstico in vitro	Per uso Diagnostica in vitro
RUO	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
REF	Catalogue number	Katalog-Nr.	Référence	Número de catálogo	No. di Cat.
LOT	Lot. No. / Batch code	Chargen-Nr.	No. de lot	Número de lote	Lotto no
Σ	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Storage Temperature	Lagerungstemperatur	Température de conservation	Temperatura de conservacion	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeits-datum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Distributed by	Distributor	Vertreiber	Distributeur	Distribuidor	Distributore
Content	Content	Inhalt	Contenu	Contenido	Contenuto
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Numéro	Volumen/Número	Volume/Quantità
Microtiterwells	Microtiterwells	Mikrotiterwells	Plaques de micro-titration	Placas multipocillo	Micropozzetti
Antiserum	Antiserum	Antiserum	Antisérum	Antisuero	Antisiero
Enzyme Conjugate	Enzyme Conjugate	Enzymkonjugat	Conjugué enzymatique	Conjugado enzimático	Tracciante enzimatico
Enzyme Complex	Enzyme Complex	Enzymkomplex	Complexe enzymatique	Complex enzimático	Complesso enzimatico
Substrate Solution	Substrate Solution	Substratlösung	Solution substrat	Solución de sustrato	Soluzione di substrato
Stop Solution	Stop Solution	Stopplösung	Solution d'arrêt	Solución de parada	Soluzione d´ arresto
Zero Standard	Zero Standard	Nullstandard	Zero Standard	Estándar cero	Standard zero
Standard	Standard	Standard	Standard	Estándar	Standard
Control	Control	Kontrolle	Contrôle	Control	Controllo
Assay Buffer	Assay Buffer	Assaypuffer	Tampon d'essai	Tampón de ensayo	Tampone del test
Wash Solution	Wash Solution	Waschlösung	Solution de lavage	Solución de lavado	Soluzione di lavaggio
1N NaOH	1N NaOH	1N NaOH	1N NaOH	1N NaOH	1N NaOH (idrossido di sodio 1N)
1 N HCl	1 N HCl	1 N HCl	1N HCl	1 N HCl	
Sample Diluent	Sample Diluent	Probenverdünnungs-medium	Solution pour dilution de l'échantillon	Solución para dilución de la muestra	Diluente dei campioni
Conjugate Diluent	Conjugate Diluent	Konjugatverdünnungs-medium	Solution pour dilution du conjugué	Solución para dilución del conjugado	Diluente del tracciante

Monobind Inc.
Lake Forest, CA 92630, USA

AccuBind
ELISA Microwells

Calcitonin Test System
Product Code: 9325-300

1.0 INTRODUCTION

Intended Use: The Quantitative Determination of Calcitonin Concentration in Human Serum by a Microplate Enzyme Immunoassay, Colorimetric

2.0 SUMMARY AND EXPLANATION OF THE TEST

Calcitonin is a 32 amino acid alpha helix produced by the follicular cells of the thyroid gland. A cleavage product of procalcitonin (PCT), calcitonin is a product of the CALC1 gene in humans and provides support in regulating calcium homeostasis, lowering serum calcium concentrations and preventing hypercalcemia. Calcitonin is characterized by an N-terminal disulfide bridge, which contributes to its biological activity, and a C-terminal proline residue.

Calcitonin plays a role in calcium metabolism, with osteoclasts the most significant homeostatic targets. Calcitonin binds to CT receptors (CTRs) on osteoclasts, halting calcium resorption via prevention of cell differentiation and motility. CTR receptors are also found in the kidneys and hypothalamus, providing an excretion route for excess serum calcium. Calcitonin modulates calcium absorption via CTR receptors on renal tubules, preventing excess calcium uptake. CTR receptors belong to the family of G-protein coupled receptors, utilizing cAMP messengers to amplify and transduce signals initiated by calcitonin-CTR binding.

Calcitonin has emerged as a therapeutic avenue for hypercalcemia patients, utilized as a biomarker PCT for its rapid biomarker for medullary carcinoma of the thyroid (MCT), providing a facile and direct measurement of carcinogenic activity. Calcitonin levels are typically low in normal populations, and elevated levels suggest the presence of hypercalcemia or potential loss of thyroid function.

Medullary thyroid carcinoma is typically associated with elevated levels of calcitonin. Parafollicular C cells containing mutations in the RET gene will display elevated expression of calcitonin and the presence of nodules in the lymph nodes, potentially disrupting calcium homeostasis.

3.0 PRINCIPLE

Sandwich Equilibrium Method (Type 2):
The calcitonin immunoassay is an adapted two-site sandwich ELISA. In this assay, standards and patient samples are simultaneously incubated with the enzyme labeled detection antibody and antibody on a coated microplate well. At the end of the assay incubation, the microwell is washed to remove unbound components and the enzyme bound to the solid phase is incubated with the substrate, tetramethylbenzidine (TMB). An acidic stopping solution is then added to stop the reaction and converts the color to yellow. The intensity of the yellow color is directly proportional to the concentration of calcitonin in the sample. Standards are used to generate a dose response curve of absorbance unit vs. concentration. Concentrations of calcitonin present in the controls and patient samples are determined directly from this curve.

The essential reagents required for a sandwich equilibrium assay include high affinity and specificity antibodies (signal and capture), with different and distinct epitope recognition, **in excess,** and native antigen. In this procedure, the calibrator, control or patient sample is added to the wells coated with anti-calcitonin antibody. Calcitonin from the sample binds to the anti-calcitonin (MoAb) on the wells. Subsequently an enzyme labeled anti-calcitonin is added to the wells. Calcitonin from the sample forms a sandwich between the two antibodies. Excess enzyme and sample is removed via a wash step. The interaction is illustrated by the following equation:

$$^{Enz}Ab_{(p)} + Ag_{CT} + Ab_{(m)} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} {}^{Enz}Ab_{(p)} - Ag_{CT} - Ab_{(m)}$$

$Ab_{(m)}$ = Anti-calcitonin (MoAb) (On the Microwells in Excess Quantity)
Ag_{CT} = Native Antigen (Variable Quantity)
$^{Enz}Ab_{(CT)}$ = Enzyme labeled Mouse α CT (P) (Excess Quantity)
$^{Enz}Ab_{(CT)} - Ag_{CT} - Ab_{(m)}$ = Ag-Antibodies Sandwich complex
k_a = Rate Constant of Association
k_{-a} = Rate Constant of Dissociation

The enzyme activity in the antibody-bound fraction is directly proportional to the native antigen concentration. By utilizing several different serum references of known antigen values, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

A suitable substrate is added to the wells to generate color in varying intensity depending upon the concentration of calcitonin in the wells. The intensity of the color in the sample can be visually compared to the known calibrators to obtain qualitative results or the color development can be read with the help of a microplate spectrophotometer to obtain quantitative results.

4.0 REAGENTS

Materials Provided:

A. Calcitonin Calibrators – 1.0 ml/vial (Dried) – Icons A-F
Six (6) vials of references for Calcitonin at levels of 0(**A**), 10(**B**), 40(**C**), 150(**D**), 400(**E**) and 1000(**F**) pg/ml. Store at 2-8°C. ***Reconstitute each vial with 1ml of distilled or deionized water***. The reconstituted calibrators are stable for 4 hours at 2-8°C. A preservative has been added. For longer periods after reconstitution, aliquot into smaller portions and freeze (<-20°C) for up to 3 months. Freeze and thaw cycles should be minimized to one time only.
Note: The calibrators are traceable against the WHO 1st International Standard NIBSC Code 89/620. Values in pg/ml can be converted to µIU/ml by multiplying by 0.19. For example, 40 pg/ml x 0.19 = 7.6 µIU/ml calcitonin.

B. Calcitonin Control M – 1.0 ml/vial (Dried) – Icon M
One (1) vial of reference control for Calcitonin .Store at 2-8 °C. ***Reconstitute each vial with 1ml of distilled or deionized water***. The reconstituted control is stable for 4 hours at 2-8°C. A preservative has been added. For longer periods after reconstitution, aliquot into smaller portions and freeze (<-20°C) for up to 3 months. Freeze and thaw cycles should be minimized to one time only.

C. Calcitonin Enzyme Reagent – 6 ml/vial – Icon
One (1) vial containing anti-Calcitonin monoclonal antibody coupled to HRP (horseradish peroxidase) in a protein-based buffer and a non-mercury preservative. Store at 2-8°C.

D. PCT Antibody Coated Plate – 96 wells – Icon
One 96-well microplate coated with anti-PCT/Calcitonin monoclonal antibody, packaged in an aluminum bag with a drying agent. Store at 2-8°C.

E. Wash Solution Concentrate – 20 ml/vial – Icon
One (1) vial containing a surfactant in buffered saline. A preservative has been added. Store at 2-8°C.

F. Substrate Reagent – 12 ml/vial – Icon S^N
One (1) vial containing tetramethylbenzidine (TMB) and hydrogen peroxide in buffer. Store at 2-8°C.

G. Stop Solution – 8 ml/vial – Icon
One (1) vial containing a strong acid (0.5M H_2SO_4). Store at 2-8°C.

H. Product Instructions.

Note 1: Do not use reagents beyond the kit expiration date.
Note 2: Do not expose reagents to heat, sun, or strong light. **Opened reagents are stable for sixty (60) days when stored at 2-8°C, unless otherwise specified. Kit and component stability are identified on label.**
Note 3: The above components are for a single 96-well microplate. For other kit configurations, refer to table at the end of insert.

4.1 Required But Not Provided:
1. Pipette capable of delivering 0.050ml (50µl) volumes with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.050ml (50µl), 0.100ml (100µl), and 0.350ml (350µl) volumes with a precision of better than 1.5%.
3. Microplate washers or a squeeze bottle (optional).
4. Microplate Reader with 450nm and 620nm wavelength absorbance capability.
5. Absorbent paper for blotting the microplate wells.
6. Plastic wrap or microplate covers for incubation steps.
7. Vacuum aspirator (optional) for wash steps.
8. Timer.
9. Quality control materials.

5.0 PRECAUTIONS

For In Vitro Diagnostic Use
Not for Internal or External Use in Humans or Animals

All products that contain human serum have been found to be non-reactive for Hepatitis B Surface Antigen, HIV 1&2 and HCV Antibodies by FDA licensed reagents. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Edition, 1988, HHS Publication No. (CDC) 88-8395.

Safe disposal of kit components must be according to local regulatory and statutory requirement.

6.0 SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood serum in type and the usual precautions in the collection of venipuncture samples should be observed. For accurate comparison to established normal values, a fasting morning serum sample should be obtained. The blood should be collected in a plain redtop venipuncture tube without additives or anti-coagulants. Allow the blood to clot for samples. Centrifuge the specimen to separate the serum from the cells.

Samples may be refrigerated at 2-8 °C for a maximum period of 24 hours. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20 °C for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.100ml (100µl) of the specimen is required.

7.0 QUALITY CONTROL

Each laboratory should assay controls at levels in the low, medium and high ranges of the dose response curve for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

8.0 REAGENT PREPARATION

1. Wash Buffer
Dilute contents of wash solution concentrate to 1000ml with distilled or deionized water in a suitable storage container. Diluted buffer can be stored at 2-30°C for up to 60 days.

9.0 TEST PROCEDURE

Before proceeding with the assay, bring all reagents, serum reference calibrators and controls to room temperature (20-27°C).
*****Test Procedure should be performed by a skilled individual or trained professional*****

1. Format the microplates' wells for each serum reference calibrator, control and patient specimen to be assayed in duplicate. **Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.**
2. Pipette 0.050 ml (50 µl) of the appropriate serum reference calibrator, control or specimen into the assigned well.
3. Add 0.050 ml (50 µl) of the Enzyme Reagent to each well. **It is very important to dispense all reagents close to the bottom of the coated well.**
4. Swirl the microplate gently for 20-30 seconds to mix (500 – 600 rpm) and cover.
5. Incubate 60 minutes (1 hour) at room temperature.
6. Discard the contents of the microplate by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper.
7. Add 0.350 ml (350 µl) of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.**
8. Add 0.100 ml (100 µl) of Substrate Reagent to all wells. **Always add reagents in the same order to minimize reaction time differences between wells.**
   DO NOT SHAKE PLATE AFTER SUBSTRATE ADDITION
9. Incubate at room temperature for twenty (20) minutes.
10. Add 0.050 ml (50 µl) of stop solution to each well and mix gently for 15-20 seconds. **Always add reagents in the same order to minimize reaction time differences between wells.**
11. Read the absorbance in each well at 450nm (using a reference wavelength of 630nm to minimize well imperfections) in a microplate reader. **The results should be read within fifteen (15) minutes of adding the stop solution.**

Note 1: **For re-assaying specimens with concentrations greater than 1000 pg/ml, dilution should be performed.**
Note 2: **Do not use reagents that are contaminated or have bacterial growth.**
Note 3: **Cycle (start and stop) mixing (4 cycles) for 5-8 seconds/cycle is more efficient than one continuous (20-30 seconds) cycle to achieve homogeneity. A plate mixer can be used to perform the mixing cycles.**
Note 3: **It is extremely important to accurately dispense the correct volume with a calibrated pipette and by adding near the bottom of the microwells at an angle while touching the side of the well.**

10.0 CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of Calcitonin in unknown specimens.
1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the absorbance for each duplicate calibrator versus the corresponding calcitonin concentration in pg/ml on linear graph paper.
3. Connect the points with a best-fit curve.
4. To determine the concentration of calcitonin for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in pg/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated).

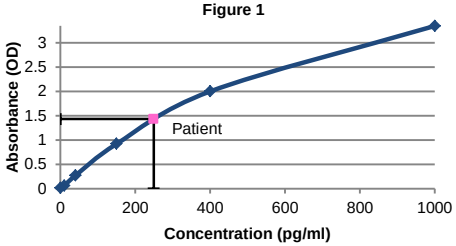
Note: Computer data reduction software designed for ELISA assays may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained.

EXAMPLE 1

Sample I.D.	Conc. (pg/ml)	Mean Abs
Cal A	0	0.016
Cal B	10	0.062
Cal C	40	0.268
Cal D	150	0.772
Cal E	400	2.150
Cal F	1000	3.347
Control M	80	0.365

*The data presented in Example 1 and Figure 1 is for illustration only and **should not** be used in lieu of a dose response curve prepared with each assay.

Figure 1



*If the absorbance readout is off-scale or higher than the average absorbance of the highest calibrator, sample should be repeated with dilution.

11.0 Q.C. PARAMETERS

In order for the assay results to be considered valid the following criteria should be met:

1. The absorbance (OD) of calibrator F (1000 pg/ml) should be ≥1.3
2. Four out of six quality control pools should be within the established ranges.

12.0 RISK ANALYSIS

The MSDS and Risk Analysis Form for this product are available on request from Monobind Inc.

12.1 Assay Performance

1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. The addition of substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the substrate and stop solution should be added in the same sequence to eliminate any time-deviation during reaction.
6. Plate readers measure vertically. Do not touch the bottom of the wells.
7. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
8. Use components from the same lot. No intermixing of reagents from different batches.
9. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from Monobind's IFU may yield inaccurate results.
10. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.
11. It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.
12. Risk Analysis- as required by CE Mark IVD Directive 98/79/EC - for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com.

12.2 Interpretation

1. ***Measurements and interpretation of results must be performed by a skilled individual or trained professional.***
2. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.
3. The reagents for the test system procedure have been formulated to eliminate maximal interference; however, potential interaction between rare serum specimens and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problematic for all kinds of immunoassays. (*Boscato LM Stuart MC. 'Heterophilic antibodies: a problem for all immunoassays' Clin.Chem. 1988:3427-33).* For diagnostic purposes, the results from this assay should be used in combination with clinical examination, patient history, and all other clinical findings.
4. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
5. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, Monobind shall have no liability.
6. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.
7. The Calcitonin ELISA kit has exhibited no high dose hook effect with samples spiked with 1,000,000 pg/ml of calcitonin. Samples with calcitonin levels greater than the highest calibrator, however, should be diluted and re-assayed for correct values.

13.0 EXPECTED RANGES OF VALUES

Calcitonin levels were measured in apparently normal individuals (N=246). The values obtained ranged from 0.455 to 12.932 pg/ml. Based on statistical tests for skewness and kurtosis, the population follows the normal or Gaussian distribution as shown in histograms. The geometric mean ± 2 standard deviations of the mean (>95% Confidence) were calculated and found to be 6.2 ± 5.6 pg/ml.

It is important to keep in mind that establishment of a range of values, which can be expected to be found by a given method for a population of "normal" persons, is dependent upon a multiplicity of factors: the specificity of the method, the population tested and the precision of the method in the hands of the analyst. For these reasons, each laboratory should depend upon the range of expected values established by the Manufacturer only until an in-house range can be determined by the analysts using the method with a population indigenous to the area in which the laboratory is located.

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

The within and between assay precisions of the Calcitonin AccuBind® ELISA test system were determined by analysis of three different levels of pool control sera. The number (N), mean (X) value, standard deviation (σ) and coefficient of variation (C.V.) of each of these control sera are presented in Table 1 and Table 2.

TABLE 1
Within Assay Precision (Values in pg/ml)

Sample	N	X	σ	C.V. %
Low	20	26.23	2.58	9.9
Normal	20	65.50	3.67	5.57
High	20	318.101	7.88	2.51

TABLE 2
Between Assay Precision (Values in pg/ml)

Sample	N	X	σ	C.V.%
Low	20	26.03	3.81	14.62
Normal	20	65.97	12.24	18.55
High	20	313.73	31.02	9.89

14.2 Sensitivity

The Calcitonin AccuBind® ELISA test system has a LoB = 1.84 pg/ml and LoD = LoQ = 2.15 pg/ml.

Table: Cross-Reactivity

SUBSTANCE	Amount Tested	Cross Reactivity
Procalcitonin	100 ng/ml	<0.001
Katacalcin	25 ng/ml	<0.001
a-CGRP	30 ng/ml	<0.001
b-CGRP	30 ng/ml	<0.001
Parathyroid Hormone	10 ng/ml	<0.001

15.0 REFERENCES

1. Felsenfeld, A. J.; Levine, B.S. "Calcitonin, the forgotten hormone: does it deserve to be forgotten?" *Clin, Kidney Jour.*, 8: 180-187, 2015.
2. Mallete, L.E., Gagel, R.F.: "Parathyroid Hormone and Calcitonin". In: Murray J.F. (ed) *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism*. American Society for Bone and Mineral Research, Kelseyville; William Byrd Press, Richmond, pp. 65-69, 1990.
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Effective Date: 2023-Feb-06 Rev. 3 **DCO: 1600**
MP9325 **Product Code: 9325-300**

Size		96(A)	192(B)
Reagent (fill)	A)	1ml (Dried) set	1ml (Dried) set
	B)	1ml (Dried) set	1ml (Dried) set
	C)	1 (6ml)	2 (6ml)
	D)	1 plate	2 plates
	E)	1 (20ml)	1 (20ml)
	F)	1 (12ml)	2 (12ml)
	G)	1 (8ml)	2 (8ml)

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IVD 2°-8° CE
EC REP **CEpartner4U,** Esdoornlaan 13 3951 DBMaarn, The Neatherlands **www.cepartner4u.eu**

Please visit our website to learn more about our products and services.

Glossary of Symbols
(EN 980/ISO 15223)

- IVD — In Vitro - Diagnostic Medical Device
- 2°-8° — Temperature Limitation Storage Condition (2-8° C)
- Consult Instructions for Use
- REF — Catalogue Number
- Σ — Contains Sufficient Test for Σ
- LOT — Batch Code
- Used By (Expiration Day)
- Date of Manufacturer
- Manufacturer
- EC REP — Authorized Rep in European Country
- CE — European Conformity

Monobind Inc.
Lake Forest, CA 92630, USA

AccuBind
ELISA Microwells

Carcinoembryonic Antigen Next Generation (CEA-Next Generation) Test System

Product Code: 4625-300

1.0 INTRODUCTION

Intended Use: The Quantitative Determination of Carcinoembryonic Antigen (CEA) Concentration in Human Serum by a Microplate Enzyme Immunoassay, Colorimetric

2.0 SUMMARY AND EXPLANATION OF THE TEST

Carcinoembryonic antigen (CEA) is a glycoprotein with a molecular weight of 180 kDA. CEA is the first of the so-called carcinoembryonic proteins that was discovered in 1965 by Gold and Freeman.[1] CEA is the most widely used marker for gastrointestinal cancer.

Although CEA is primarily associated with colorectal cancers, other malignancies that can cause elevated levels of CEA include breast, lung, stomach, pancreas, ovary and other organs. Benign conditions that cause significantly higher than normal levels include inflammation of lung and gastrointestinal (GI) tract and benign liver cancer.[2,3] Heavy smokers, as a group, have higher than normal baseline concentration of CEA.

In this method, CEA calibrator, patient specimen or control is first added to a streptavidin coated well. Biotinylated monoclonal and enzyme labeled antibodies (directed against distinct and different epitopes of CEA) are added and the reactants mixed. Reaction between the various CEA antibodies and native CEA forms a sandwich complex that binds with the streptavidin coated to the well.

After the completion of the required incubation period, the enzyme-CEA antibody bound conjugate is separated from the unbound enzyme-CEA conjugate by aspiration or decantation. The activity of the enzyme present on the surface of the well is quantitated by reaction with a suitable substrate to produce color.

The employment of several serum references of known carcinoembryonic antigen (CEA) levels permits the construction of a dose response curve of activity and concentration. From comparison to the dose response curve, an unknown specimen's activity can be correlated with CEA concentration.

3.0 PRINCIPLE

Immunoenzymometric assay (TYPE 3):
The essential reagents required for an immunoenzymometric assay include high affinity and specificity antibodies (enzyme and immobilized), with different and distinct epitope recognition, **in excess,** and native antigen. In this procedure, the immobilization takes place during the assay at the surface of a microplate well through the interaction of streptavidin coated on the well and exogenously added biotinylated monoclonal anti-CEA antibody.

Upon mixing monoclonal biotinylated antibody, the enzyme-labeled antibody and a serum containing the native antigen, reaction results between the native antigen and the antibodies, without competition or steric hindrance, to form a soluble sandwich complex. The interaction is illustrated by the following equation:

$$^{Enz}Ab + Ag_{CEA} + {}^{Btn}Ab_{(m)} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} {}^{Enz}Ab - Ag_{CEA} - {}^{Btn}Ab_{(m)}$$

$^{Btn}Ab_{(m)}$ = Biotinylated Monoclonal Antibody (Excess Quantity)
Ag_{CEA} = Native Antigen (Variable Quantity)
^{Enz}Ab = Enzyme labeled Antibody (Excess Quantity)
$^{Enz}Ab - Ag_{CEA} - {}^{Btn}Ab_{(m)}$ = Antigen-Antibodies Sandwich Complex
k_a = Rate Constant of Association
k_{-a} = Rate Constant of Dissociation

Simultaneously, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antibody. This interaction is illustrated below:
$^{Enz}Ab - Ag_{CEA} - {}^{Btn}Ab_{(m)} + Streptavidin_{C.W.} \Rightarrow$ Immobilized complex
$Streptavidin_{C.W.}$ = Streptavidin immobilized on well
Immobilized complex = sandwich complex bound to the well

After equilibrium is attained, the antibody-bound fraction is separated from unbound antigen by decantation or aspiration. The enzyme activity in the antibody-bound fraction is directly proportional to the native antigen concentration. By utilizing several different serum references of known antigen values, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

4.0 REAGENTS

Materials Provided:

A. CEA Next Generation Calibrators – 1ml/vial Icons A-F
Six (6) vials of references CEA Antigen at levels of 0(**A**), 5(**B**), 10(**C**), 25(**D**), 100(**E**) and 250(**F**) ng/ml. A preservative has been added. Store at 2-8°C.
Note: The standards, human serum based, were calibrated using a reference preparation, which was assayed against the 1st International Reference Preparation (IRP# 73/601).

B. CEA Next Generation Enzyme Reagent -13ml/vial -Icon Ⓔ
One (1) vial containing enzyme labeled antibody, biotinylated monoclonal mouse IgG in buffer, red dye, and preservative. Store at 2-8°C.

C. Streptavidin Coated Plate – 96 wells – Icon ⇓
One 96-well microplate coated with streptavidin and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

D. Wash Solution Concentrate – 20ml/vial - Icon
One (1) vial contains a surfactant in buffered saline. A preservative has been added. Store at 2-8°C.

E. Substrate A – 7ml/vial - Icon S^A
One (1) vial contains tetramethylbenzidine (TMB) in buffer. Store at 2-8°C. See "Reagent Preparation."

F. Substrate B – 7ml/vial - Icon S^B
One (1) vial contains hydrogen peroxide (H_2O_2) in buffer. Store at 2-8°C. See "Reagent Preparation."

G. Stop Solution – 8ml/vial - Icon
One (1) vial contains a strong acid (1N HCl). Store at 2-8°C.

H. Product Instructions.

Note 1: Do not use reagents beyond the kit expiration date.
Note 2: Avoid extended exposure to heat and light. **Opened reagents are stable for sixty (60) days when stored at 2-8°C. Kit and component stability are identified on the label.**
Note 3: Above reagents are for a single 96-well microplate

4.1 Required But Not Provided:
1. Pipette(s) capable of delivering 0.025 & 0.050ml (25µl & 50µl) volumes with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100 & 0.350ml (100 & 350µl) volumes with a precision of better than 1.5%.
3. Microplate washers or a squeeze bottle (optional).
4. Microplate Reader with 450nm and 620nm wavelength absorbance capability.
5. Absorbent Paper for blotting the microplate wells.
6. Plastic wrap or microplate cover for incubation steps.
7. Vacuum aspirator (optional) for wash steps.
8. Timer.
9. Quality control materials

5.0 PRECAUTIONS

For In Vitro Diagnostic Use
Not for Internal or External Use in Humans or Animals

All products that contain human serum have been found to be non-reactive for Hepatitis B Surface Antigen, HIV 1&2 and HCV Antibodies by FDA licensed reagents. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Edition, 1988, HHS Publication No. (CDC) 88-8395.

Safe disposal of kit components must be according to local regulatory and statutory requirement.

6.0 SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood, serum in type, and the usual precautions in the collection of venipuncture samples should be observed. For accurate comparison to established normal values, a fasting morning serum sample should be obtained. The blood should be collected in a plain redtop venipuncture tube without additives or anti-coagulants. Allow the blood to clot. Centrifuge the specimen to separate the serum from the cells.

In patients receiving therapy with high biotin doses (i.e. >5mg/day), no sample should be taken until at least 8 hours after the last biotin administration, preferably overnight to ensure fasting sample.

Samples may be refrigerated at 2-8°C for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20°C for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.050ml (50µl) of the specimen is required.

7.0 QUALITY CONTROL

Each laboratory should assay controls at levels in the low, normal and elevated range for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

8.0 REAGENT PREPARATION

1. **Wash Buffer**
Dilute contents of wash concentrate to 1000ml with distilled or deionized water in a suitable storage container. Store diluted buffer at 2-30°C for up to 60 days.
2. **Working Substrate Solution –** Stable for one (1) year
Pour the contents of the amber vial labeled Solution 'A' into the clear vial labeled Solution 'B'. Place the yellow cap on the clear vial for easy identification. Mix and label accordingly. Store at 2 - 8°C.

Note 1: Do not use the working substrate if it looks blue.
Note 2: Do not use reagents that are contaminated or have bacteria growth.

9.0 TEST PROCEDURE

Before proceeding with the assay, bring all reagents, serum reference calibrators and controls to room temperature (20 -27°C).
*****Test Procedure should be performed by a skilled individual or trained professional*****

1. Format the microplates' wells for each serum reference calibrator, control and patient specimen to be assayed in duplicate. **Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.**
2. Pipette 0.025 ml (25µl) of the appropriate serum reference calibrator, control or specimen into the assigned well.
3. Add 0.100ml (100µl) of the CEA Enzyme Reagent to each well. **It is very important to dispense all reagents close to the bottom of the coated well.**
4. Swirl the microplate gently for 20-30 seconds to mix and cover.
5. Incubate 60 minutes at room temperature.
6. Discard the contents of the microplate by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper.
7. Add 0.350ml (350µl) of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.**
8. Add 0.100 ml (100µl) of working substrate solution to all wells (see Reagent Preparation Section). **Always add reagents in the same order to minimize reaction time differences between wells.**

DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION

9. Incubate at room temperature for fifteen (15) minutes.
10. Add 0.050ml (50µl) of stop solution to each well and mix gently for 15-20 seconds. **Always add reagents in the same order to minimize reaction time differences between wells.**
11. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) in a microplate reader. **The results should be read within thirty (30) minutes of adding the stop solution.**

10.0 CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of Carcinoembryonic antigen in unknown specimens.
1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the absorbance for each duplicate serum reference versus the corresponding CEA concentration in ng/ml on linear graph paper (do not average the duplicates of the serum references before plotting).
3. Draw the best-fit curve through the plotted points.
4. To determine the concentration of CEA for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in ng/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance 0.320 ng/ml intersects the dose response curve at 18.1 ng/ml CEA concentration (see Figure 1).

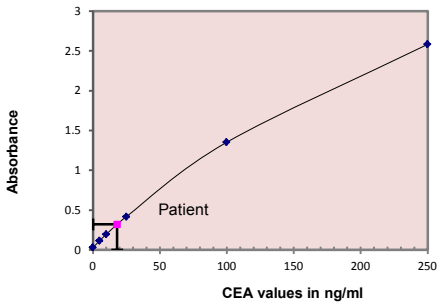
Note: Computer data reduction software designed for ELISA assays may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained.

EXAMPLE 1

Sample I.D.	Well Number	Abs (A)	Mean Abs (B)	Value (ng/ml)
Cal A	A1	0.028	0.027	0
	B1	0.026		
Cal B	C1	0.115	0.115	5
	D1	0.114		
Cal C	E1	0.196	0.196	10
	F1	0.196		
Cal D	G1	0.432	0.418	25
	H1	0.404		
Cal E	A2	1.403	1.353	100
	B2	1.303		
Cal F	C2	2.580	2.558	250
	D2	2.535		
Patient	E2	0.302	0.320	**18.1**
	F2	0.337		

*The data presented in Example 1 and Figure 1 is for illustration only and **should not** be used in lieu of a dose response curve prepared with each assay.

Figure 1



11.0 Q.C. PARAMETERS

In order for the assay results to be considered valid the following criteria should be met:

1. The absorbance (OD) of calibrator F should be ≥ 1.3
2. Four out of six quality control pools should be within the established ranges.

12.0 RISK ANALYSIS

The MSDS and Risk Analysis Form for this product are available on request from Monobind Inc.

12.1 Assay Performance

1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. The addition of substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the substrate and stop solution should be added in the same sequence to eliminate any time-deviation during reaction.
6. Plate readers measure vertically. Do not touch the bottom of the wells.
7. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
8. Use components from the same lot. No intermixing of reagents from different batches.
9. Patient specimens with CEA concentrations above 250 ng/ml may be diluted (for example 1/10 or higher) with normal male serum (CEA < 5 ng/ml) and re-assayed. The sample's concentration is obtained by multiplying the result by the dilution factor (10).
10. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from Monobind's IFU may yield inaccurate results.
11. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.
12. It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.
13. Risk Analysis- as required by CE Mark IVD Directive 98/79/EC - for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com.

12.2 Interpretation

1. ***Measurements and interpretation of results must be performed by a skilled individual or trained professional.***
2. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.
3. The reagents for the test system procedure have been formulated to eliminate maximal interference; however, potential interaction between rare serum specimens and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for all kinds of immunoassays. (Boscato LM Stuart MC. 'Heterophilic antibodies: a problem for all immunoassays' Clin.Chem. 1988:3427-33). For diagnostic purposes, the results from this assay should be used in combination with clinical examination, patient history and all other clinical findings.
4. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
5. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, Monobind shall have no liability.
6. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.
7. CEA has a low clinical sensitivity and specificity as a tumor marker. Clinically an elevated **CEA value alone is not of diagnostic value as a test for cancer** and should only be used in conjunction with other clinical manifestations (observations) and diagnostic parameters. There are patients with colorectal cancer that do not exhibit elevated CEA values and elevated CEA values do not always change with progression or regression of disease. Smokers demonstrate a higher range of baseline values than non-smokers.

13.0 EXPECTED RANGES OF VALUES

Nearly 99% of non-smokers have CEA concentrations less than 5ng/ml. Similarly 99% of smokers have concentrations less than 10ng/ml.[4]

TABLE I
Expected Values for the CEA Next Generation AccuBind® ELISA Test System

Non-smokers	<5ng/ml
Smokers	<10ng/ml

It is important to keep in mind that establishment of a range of values, which can be expected to be found by a given method for a population of "normal" persons, is dependent upon a multiplicity of factors: the specificity of the method, the population tested and the precision of the method in the hands of the analyst. For these reasons, each laboratory should depend upon the range of expected values established by the Manufacturer only until an in-house range can be determined by the analysts using the method with a population indigenous to the area in which the laboratory is located.

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

The within and between assay precisions of the CEA Next Generation AccuBind® ELISA test system were determined by analyses on three different levels of control sera. The number (N), mean value (X), standard deviation (σ) and coefficient of variation (C.V.) for each of these control sera are presented in Table 2 and Table 3.

TABLE 2
Within Assay Precision (Values in ng/ml)

Sample	N	X	σ	C.V.
Level 1	20	2.6	0.25	9.6%
Level 2	20	12.5	1.01	8.1%
Level 3	20	24.1	1.35	5.6%

TABLE 3
Between Assay Precision* (Values in ng/ml)

Sample	N	X	σ	C.V.
Level 1	10	2.8	0.30	10.7%
Level 2	10	12.8	1.18	9.2%
Level 3	10	23.5	1.85	7.8%

*As measured in ten experiments in duplicate.

14.2 Sensitivity

The CEA Next Generation AccuBind® ELISA test system has a sensitivity of 0.025 ng. This is equivalent to a sample containing 1 ng/ml CEA concentration. The sensitivity was ascertained by determining the variability of the '0 ng/ml' calibrator and using the 2σ (95% certainty) statistic to calculate the minimum dose.

14.3 Accuracy

The CEA Next Generation AccuBind® ELISA method was compared with a reference method. Biological specimens from normal and elevated concentrations were assayed. The total number of such specimens was 64. The values ranged from 0.4 – 128ng/ml. The least square regression equation and the correlation coefficient were computed for the CEA Next Generation AccuBind® ELISA method in comparison with the reference method. The data obtained is displayed in Table 4.

TABLE 4

Method	Mean	Least Square Regression Analysis	Correlation Coefficient
Monobind (X)	10.01	y = 1.17+0.977x	0.995
Reference (Y)	9.04		

E. Specificity:

Highly specific antibodies to CEA molecules have been used in the CEA Next Generation AccuBind® ELISA test system. No interference was detected with the performance of CEA Next Generation AccuBind® ELISA upon addition of massive amounts of the following substances to a human serum pool.

Substance	Concentration
Acetylsalicylic Acid	100 µg/ml
Ascorbic Acid	100 µg/ml
Caffeine	100 µg/ml
AFP	10 µg/ml
PSA	1.0 µg/ml
CA-125	10,000 U/ml
hCG	1000 IU/ml
hLH	10 IU/ml
hTSH	100 mIU/ml
hPRL	100 µg/ml

14.5 Linearity & Hook Effect:

Three different lot preparations of the CEA Next Generation AccuBind® ELISA reagents were used to assess the linearity and hook effect. Massive concentrations of CEA (> 60,000 ng/ml) were used for linear dilutions in pooled human patient sera.

The test showed no hook effect up to concentrations of 60,000 ng/ml and a within dose recovery of 92.0 to 111.4%.

15.0 REFERENCES

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Revision: 3 **Date: 2019-Jul-16** **DCO: 1353**
MP4625 **Product Code: *4625-300***

Size		96(A)	192(B)
Reagent (fill)	A)	1ml set	1ml set
	B)	1 (13ml)	2 (13ml)
	C)	1 plate	2 plates
	D)	1 (20ml)	1 (20ml)
	E)	1 (7ml)	2 (7ml)
	F)	1 (7ml)	2 (7ml)
	G)	1 (8ml)	2 (8ml)

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IVD 2° 8° CE
EC REP **CEpartner4U,** Esdoornlaan 13
3951 DBMaarn, The Neatherlands
www.cepartner4u.eu

Please visit our website to learn more about our products and services.

Glossary of Symbols
(EN 980/ISO 15223)

IVD — In Vitro - Diagnostic Medical Device	2° 8° — Temperature Limitation Storage Condition (2-8° C)	Consult Instructions for Use
REF — Catalogue Number	Σ — Contains Sufficient Test for Σ	LOT — Batch Code
Used By (Expiration Day)	Date of Manufacturer	Manufacturer
EC REP — Authorized Rep in European Country	CE — European Conformity	

Monobind Inc.
Lake Forest, CA 92630, USA

AccuBind
ELISA Microwells

Cortisol Test System
Product Code: 3625-300

1.0 INTRODUCTION

Intended Use: The Quantitative Determination of Total Cortisol Concentration in Human Serum or Plasma by a Microplate Enzyme Immunoassay, Colorimetric

2.0 SUMMARY AND EXPLANATION OF THE TEST

Cortisol (hydrocortisone, compound F) is the most potent glucocorticoid produced by the human adrenal cortex. As with other adrenal steroids, cortisol is synthesized from cholesterol, through a series of enzymatically mediated steps, by the adrenal cortex.[1,2] The first and rate-limiting step in adrenal steroidogenesis, conversion of cholesterol to pregnenolone, is stimulated by pituitary adrenocorticotropic hormone (ACTH) which is, in turn, regulated by hypothalamic corticotropin releasing factor (CRF). ACTH and CRF secretion are inhibited by high cortisol levels. In plasma, the major portion of cortisol is bound with high affinity to corticosteroid-binding globulin (CBG, transcortin), with most of the remainder loosely bound to albumin. Physiologically effective in anti-inflammatory activity and blood pressure maintenance, cortisol is also involved in gluconeogenesis. Cortisol acts through specific intracellular receptors and has effects in numerous other physiologic systems, including immune function, glucose-counter regulation, vascular tone, substrate utilization and bone metabolism.[1-3] Cortisol is excreted primarily in urine in an unbound (free) form.

Cortisol production has an ACTH-dependent circadian rhythm with peak levels in the early morning and a nadir at night. The factors controlling this circadian rhythm are not completely defined. The circadian rhythm of ACTH/cortisol secretion matures gradually during early infancy, and is disrupted in a number of physical and psychological conditions.[4] Furthermore, increased amounts of ACTH and cortisol are secreted independently of the circadian rhythm in response to physical and psychological stress.[4,5]

Elevated cortisol levels and lack of diurnal variation have been identified in patients with Cushing's disease (ACTH hyper secretion).[2,6] Elevated circulating cortisol levels have also been identified in patients with adrenal tumors.[7] Low cortisol levels are found in primary adrenal insufficiency (e.g. adrenal hypoplasia, congenital adrenal hyperplasia, Addison's disease) and in ACTH deficiency.[1,2,8,9] Due to the normal circadian variation of cortisol levels, distinguishing normal and abnormally low cortisol levels can be difficult. Therefore, various tests to evaluate the pituitary-adrenal (ACTH-cortisol) axis, including insulin-induced hypoglycemia, short- and long-term ACTH stimulation, CRF stimulation and artificial blockage of cortisol synthesis with metronome have been performed.[8-10] Cortisol response characteristics for each of these procedures have been reported.

The Monobind Cortisol EIA Kit uses a specific monoclonal anti-cortisol antibody, and does not require prior sample extraction of serum or plasma. Cross-reactivity to other naturally-occurring steroids is low.

The employment of several serum references of known cortisol concentration permits construction of a graph of activity and concentration. From comparison to the dose response curve, an unknown specimen's activity can be correlated with cortisol concentration.

3.0 PRINCIPLE

Competitive Enzyme Immunoassay (TYPE 7):
The essential reagents required for an enzyme immunoassay include antibody, enzyme-antigen conjugate and native antigen. Upon mixing biotinylated antibody, enzyme-antigen conjugate and a serum containing the native antigen, a competition reaction results between the native antigen and the enzyme-antigen conjugate for a limited number of antibody binding sites. The interaction is illustrated by the followed equation:

$$^{Enz}Ag + Ag + Ab_{Btn} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} AgAb_{Btn} + {}^{Enz}AgAb_{Btn}$$

Ab_{Btn} = Biotinylated Antibody (Constant Quantity)
Ag = Native Antigen (Variable Quantity)
^{Enz}Ag = Enzyme-antigen Conjugate (Constant Quantity)
$AgAb_{Btn}$ = Antigen-Antibody Complex
$^{Enz}AgAb_{Btn}$ = Enzyme-antigen Conjugate -Antibody Complex
k_a = Rate Constant of Association
k_{-a} = Rate Constant of Disassociation
$K = k_a / k_{-a}$ = Equilibrium Constant

A simultaneous reaction between the biotin attached to the antibody and the streptavidin immobilized on the microwell occurs. This effects the separation of the antibody bound fraction after decantation or aspiration.
$Ab_{Btn} + {}^{Enz}AgAb_{Btn} + Streptavidin_{CW} \Rightarrow$ immobilized complex
$Streptavidin_{CW}$ = Streptavidin immobilized on well
Immobilized complex = sandwich complex bound to the solid surface

The enzyme activity in the antibody-bound fraction is inversely proportional to the native antigen concentration. By utilizing several different serum references of known antigen concentration, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

4.0 REAGENTS

Materials Provided:

A. Cortisol Calibrators – 1ml/vial – Icons A-F
Six (6) vials of serum reference for Cortisol at concentrations of 0 (**A**), 1.0 (**B**), 4.0 (**C**), 10.0 (**D**), 20.0 (**E**) and 50.0 (**F**) µg/dl. Store at 2-8°C. A preservative has been added.

B. Cortisol Enzyme Reagent – 7.0 ml/vial – Icon E
One (1) ready to use vial containing Cortisol (Analog)-horseradish peroxides (HRP) conjugate in a protein stabilizing matrix with buffer, red dye, preservative and binding protein inhibitors. Store at 2-8°C.

C. Cortisol Biotin Reagent – 7.0 ml – Icon ∇
One (1) vial containing anti-cortisol biotinylated mIgG conjugate in buffer, dye and preservative. Store at 2-8°C.

D. Streptavidin Coated Plate – 96 wells – Icon ⇓
One 96-well microplate coated with 1.0 µg/ml streptavidin and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

E. Wash Solution Concentrate – 20ml/vial – Icon
One (1) vial containing a surfactant in buffered saline. A preservative has been added. Store at 2-8°C.

E. Substrate A – 7ml/vial – Icon S^A
One (1) vial containing tetramethylbenzidine (TMB) in buffer. Store at 2-8°C.

F. Substrate B – 7ml/vial – Icon S^B
One (1) vial containing hydrogen peroxide (H_2O_2) in buffer. Store at 2-8°C.

G. Stop Solution – 8ml/vial – Icon STOP
One (1) vial containing a strong acid (1N HCl). Store at 2-8°C.

H. Product Instructions.

Note 1: Do not use reagents beyond the kit expiration date.
Note 2: Avoid extended exposure to heat and light. **Opened reagents are stable for sixty (60) days when stored at 2-8°C. Kit and component stability are identified on the label.**
Note 3: Above reagents are for a single 96-well microplate.

4.1 Required But Not Provided:
1. Pipette capable of delivering 0.025ml (25µl), 0.050ml, (50µl) and 0.100ml (100µl) volumes with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.050ml (50µl) 0.100ml (100µl) and 0.350ml (350µl) volumes with a precision of better than 1.5%.
3. Microplate washer or a squeeze bottle (optional).
4. Microplate Reader with 450nm and 620nm wavelength absorbance capability.
5. Absorbent Paper for blotting the microplate wells.
6. Plastic wrap or microplate covers for incubation steps.
7. Vacuum aspirator (optional) for wash steps.
8. Timer.
9. Quality control materials.

5.0 PRECAUTIONS

For In Vitro Diagnostic Use
Not for Internal or External Use in Humans or Animals

All products that contain human serum have been found to be non-reactive for Hepatitis B Surface Antigen, HIV 1&2 and HCV Antibodies by FDA required tests. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Edition, 1988, HHS Publication No. (CDC) 88-8395.

Safe Disposal of kit components must be according to local regulatory and statutory requirement.

6.0 SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood; serum or plasma in type and the usual precautions in the collection of venipuncture samples should be observed. For accurate comparison to established normal values, a fasting morning serum sample should be obtained. The blood should be collected in a plain redtop venipuncture tube without additives or anti-coagulants (for serum) or evacuated tube(s) containing EDTA or heparin. Allow the blood to clot for serum samples. Centrifuge the specimen to separate the serum or plasma from the cells.

In patients receiving therapy with high biotin doses (i.e. >5mg/day), no sample should be taken until at least 8 hours after the last biotin administration, preferably overnight to ensure fasting sample.

Samples may be refrigerated at 2-8°C for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20°C for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.050ml (50µl) of the specimen is required.

7.0 QUALITY CONTROL

Each laboratory should assay controls at levels in the low, normal and high range for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. The individual laboratory should set acceptable assay performance limits. In addition, maximum absorbance should be consistent with past experience. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

8.0 REAGENT PREPARATION

1. **Wash Buffer**
Dilute contents of wash solution to 1000ml with distilled or deionized water in a suitable storage container. Diluted reagent can be stored at 2-30°C for up to 60 days.

2. **Working Substrate Solution –** Stable for 1 year
Pour the contents of the amber vial labeled Solution 'A' into the clear vial labeled Solution 'B'. Place the yellow cap on the clear vial for easy identification. Mix and label accordingly. Store at 2 - 30°C.

Note 1: Do not use the working substrate if it looks blue.
Note 2: Do not use reagents that are contaminated or have bacteria growth.

9.0 TEST PROCEDURE

Before proceeding with the assay, bring all reagents, serum reference calibrators and controls to room temperature (20-27°C).
*****Test Procedure should be performed by a skilled individual or trained professional*****

1. Format the microplates' wells for each serum reference, control and patient specimen to be assayed in duplicate. **Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.**
2. Pipette 0.025 ml (25µL) of the appropriate serum reference, control or specimen into the assigned well.
3. Add 0.050 ml (50µl) of the ready to use Cortisol Enzyme Reagent to all wells
4. Swirl the microplate gently for 20-30 seconds to mix.
5. Add 0.050 ml (50µl) of Cortisol Biotin Reagent to all wells.
6. Swirl the microplate gently for 20-30 seconds to mix.
7. Cover and incubate for 60 minutes at room temperature.
8. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.
9. Add 0.350ml (350µl) of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.**
10. Add 0.100 ml (100µl) of working substrate solution to all wells (see Reagent Preparation Section). **Always add reagents in the same order to minimize reaction time differences between wells.**
DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION
11. Incubate at room temperature for fifteen (15) minutes.
12. Add 0.050ml (50µl) of stop solution to each well and gently mix for 15-20 seconds. **Always add reagents in the same order to minimize reaction time differences between wells.**
13. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) in a microplate reader. **The results should be read within thirty (30) minutes of adding the stop solution.**

Note: Dilute the samples suspected of concentrations higher than 50 µg/dl 1:5 and 1:10 with cortisol '0' µg/dl patient serum.

10.0 CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of cortisol in unknown specimens.
1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the absorbance for each duplicate serum reference versus the corresponding cortisol concentration in µg/dl on linear graph paper (do not average the duplicates of the serum references before plotting).
3. Connect the points with a best-fit curve.
4. To determine the concentration of cortisol for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in µg/dl) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance (1.071) intersects the dose response curve at (10.2 µg/dl) cortisol concentration (See Figure 1).

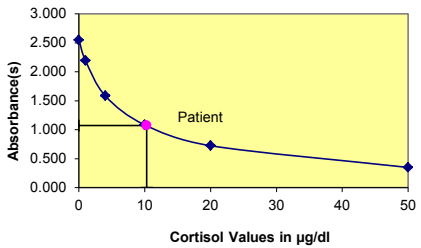
Note: Computer data reduction software designed for ELISA assays may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained.

EXAMPLE 1

Sample I.D.	Well Number	Abs (A)	Mean Abs (B)	Value (µg/dl)
Cal A	A1	2.483	2.543	0
	B1	2.575		
Cal B	C1	2.150	2.194	1.0
	D1	2.186		
Cal C	E1	1.573	1.585	4.0
	F1	1.597		
Cal D	G1	1.103	1.084	10
	H1	1.065		
Cal E	A2	0.726	0.725	20
	B2	0.724		
Cal F	C2	0.347	0.350	50
	D2	0.353		
Ctrl 1	E2	1.624	1.617	**3.74**
	F2	1.611		
Ctrl 2	G2	0.770	0.760	**18.57**
	H2	0.749		
Patient	A3	1.056	1.071	**10.24**
	B3	1.086		

*The data presented in Example 1 and Figure 1 is for illustration only and **should not** be used in lieu of a standard curve prepared with each assay.

Figure 1



11.0 Q.C. PARAMETERS

In order for the assay results to be considered valid the following criteria should be met:

1. The absorbance (OD) of calibrator 0 µg/dl should be ≥ 1.3.
2. Four out of six quality control pools should be within the established ranges.

12.0 RISK ANALYSIS

The MSDS and Risk Analysis Form for this product are available on request from Monobind Inc.

12.1 Assay Performance

1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. The addition of substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the substrate and stop solution should be added in the same sequence to eliminate any time-deviation during reaction.
6. Plate readers measure vertically. Do not touch the bottom of the wells.
7. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
8. Use components from the same lot. No intermixing of reagents from different batches.
9. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from Monobind's IFU may yield inaccurate results.
10. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.
11. It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.
12. Risk Analysis- as required by CE Mark IVD Directive 98/79/EC - for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com.

12.2 Interpretation

1. ***Measurements and interpretation of results must be performed by a skilled individual or trained professional.***
2. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.
3. The reagents for the procedure have been formulated to eliminate maximal interference; however, potential interaction between rare serum specimens and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for all kinds of immunoassays. *(Boscato LM Stuart MC.'Heterophilic antibodies: a problem for all immunoassays' Clin. Chem 1988:3427-33).* For diagnostic purposes the results from this assay should be used in combination with clinical examination, patient's history and, all other clinical findings.
4. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
5. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, Monobind shall have no liability.
6. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.
7. Total serum cortisol values may be dependent upon conditions such as time of the day for sampling or administration of prednisolone or prednisone (structurally related to cortisol). Caution must be exercised while interpreting cortisol levels for patients undergoing therapy with these and other structurally related corticosteroids such as cortisone or corticosterone.

13.0 EXPECTED RANGES OF VALUES

A study of normal adult population was undertaken to determine expected values for the Cortisol AccuBind® ELISA Test System. The mean (R) values, standard deviations (**σ**) and expected ranges (±2**σ**) are presented in Table 1.

TABLE I
Expected Values for the cortisol EIA Test System (in µg/dl)

Population	Morning	Afternoon
Adult	5 - 23 µg/dl	3 -13 µg/dl
Child	3 - 21 µg/dl	3 -10 µg/dl
Newborn	1 - 24 µg/dl	

Please note: Normal results may vary from lab to lab

It is important to keep in mind that establishment of a range of values which can be expected to be found by a given method for a population of "normal"-persons is dependent upon a multiplicity of factors: the specificity of the method, the population tested and the precision of the method in the hands of the analyst. For these reasons each laboratory should depend upon the range of expected values established by the Manufacturer only until an in-house range can be determined by the analysts using the method with a population indigenous to the area in which the laboratory is located.

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

The within and between assay precision of the Cortisol AccuBind® ELISA Test System were determined by analyses on three different levels of pool control sera. The number, mean values, standard deviation and coefficient of variation for each of these control sera are presented in Table 2 and Table 3.

TABLE 2
Within Assay Precision (Values in µg/dl)

Sample	N	X	σ	C.V.
Low	16	3.4	0.28	8.2%
Normal	16	14.2	0.91	6.4%
High	16	36.5	2.23	6.1%

TABLE 3
Between Assay Precision (Values in µg/dl)

Sample	N	X	σ	C.V.
Low	10	3.1	0.30	9.7%
Normal	10	15.1	1.06	7.0%
High	10	37.4	2.71	7.3%

*As measured in ten experiments in duplicate over a ten day period.

14.2 Sensitivity

The Cortisol AccuBind® ELISA Test System has a sensitivity of 91.5 pg. This is equivalent to a sample containing a concentration of 0.366 µg/dl. The sensitivity was ascertained by determining the variability of the 0 µg/dl serum calibrator and using the 2σ (95% certainty) statistic to calculate the minimum dose.

14.3 Accuracy

The Cortisol AccuBind® ELISA Test System was compared with a coated tube radioimmunoassay method. Biological specimens from low, normal and high cortisol level populations were used. The values ranged from 0.4 µg/dl – 95µg/dl. The total number of such specimens was 202. The least square regression equation and the correlation coefficient were computed for this cortisol EIA in comparison with the reference method. The data obtained is displayed in Table 4.

TABLE 4

Method	Mean (x)	Least Square Regression Analysis	Correlation Coefficient
Monobind (y)	16.6	y = -0.228+1.0186(x)	0.984
Reference (X)	16.8		

Only slight amounts of bias between this method and the reference method are indicated by the closeness of the mean values. The least square regression equation and correlation coefficient indicates excellent method agreement.

14.4 Specificity

The % cross-reactivity of the cortisol antibody to selected substances was evaluated by adding the interfering substance to a serum matrix at various concentrations. The cross-reactivity was calculated by deriving a ratio between doses of interfering substance to dose of cortisol needed to displace the same amount of labeled analog.

Substance	Cross Reactivity
Cortisol	1.0000
Androstenedione	0.0004
Cortisone	0.2300
Corticosterone	0.1800
11-Deoxycortisol	0.0550
Dexamethasone	0.0001
Progesterone	0.0002
17α-OH Progesterone	ND
DHEA	ND
Estradiol	ND
Estrone	ND
Danazol	ND
Testosterone	ND

15.0 REFERENCES

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Effective Date: 2019-Jul-16 Rev. 4 **DCO: 1353**
MP3625 **Product Code: 3625-300**

Size		96(A)	192(B)
Reagent (fill)	A)	1ml set	1ml set
	B)	1 (7ml)	2 (7ml)
	C)	1 (7ml)	2 (7ml)
	D)	1 plate	2 plates
	E)	1 (20ml)	1 (20ml)
	F)	1 (7ml)	2 (7ml)
	G)	1 (7ml)	2 (7ml)
	H)	1 (8ml)	2 (8ml)

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IVD 2°-8° CE
EC REP **CEpartner4U,** Esdoornlaan 13
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www.cepartner4u.eu

Please visit our website to learn more about our products and services.

Glossary of Symbols
(EN 980/ISO 15223)

IVD — In Vitro - Diagnostic Medical Device
2°-8° — Temperature Limitation Storage Condition (2-8° C)
Consult Instructions for Use
REF — Catalogue Number
Σ — Contains Sufficient Test for Σ
LOT — Batch Code
Used By (Expiration Day)
Date of Manufacturer
Manufacturer
EC REP — Authorized Rep in European Country
CE — European Conformity

Monobind Inc.
Lake Forest, CA 92630, USA

AccuBind
ELISA Microwells

C-Peptide Test System
Product Code: 2725-300

1.0 INTRODUCTION

Intended Use: The Quantitative Determination of Circulating C-Peptide Concentrations in Human Serum by a Microplate Enzyme Immunoassay, Colorimetric

2.0 SUMMARY AND EXPLANATION OF THE TEST

Diabetes is one of the leading causes of disability and death in the U.S. It affects an estimated 16 million Americans, about one third of them do not even know they have the disease. The causes of diabetes are not precisely known, but both genetic and environmental factors play a significant role. The disease is marked by deficiencies in the body's ability to produce and properly use insulin. The most common forms of diabetes are type 1, in which the body's ability to produce insulin is destroyed, and type 2, in which the body is resistant to insulin even though some amount of insulin may be produced.

In-vitro determination of insulin and *C-Peptide* levels help in the differential diagnosis of liver disease, acromegaly, Cushing's syndrome, familial glucose intolerance, insulinoma, renal failure, ingestion of accidental oral hypoglycemic drugs or insulin induced factitious hypoglycemia. Both insulin and *C-Peptide* are produced by enzymatic cleavage of proinsulin. Proinsulin is stored in the secretory granules of pancreatic β-cells and is split into a 31 amino acid connecting peptide (*C-Peptide;* MW 3600) and insulin (MW 6000). *C-Peptide* is devoid of any biological activity but appears to be necessary to maintain the structural integrity of insulin. Although insulin and *C-Peptide* are secreted into portal circulation in equimolar concentrations, fasting levels of *C-Peptide* are 5-10 fold higher than those of insulin owing to the longer half-life of *C-Peptide*. The liver does not extract *C-Peptide* however; it is removed from the circulation by degradation in the kidneys with a fraction passing out unchanged in urine. Hence urine *C-Peptide* levels correlate well with fasting *C-Peptide* levels in serum. The glucagon stimulated *C-Peptide* determination is often used for differential diagnosis of insulin-dependent from non-insulin-dependent diabetic patients.

3.0 PRINCIPLE

Immunoenzymometric assay (TYPE 3):
The essential reagents required for an immunoenzymometric assay include high affinity and specificity antibodies (Ab). (enzyme conjugated and immobilized), with different and distinct epitope recognition, **in excess,** and native antigen (Ag). In this procedure, the immobilization takes place during the assay at the surface of a microplate well through the interaction of streptavidin coated on the well and exogenously added biotinylated monoclonal C-peptide antibody.

Upon mixing monoclonal biotinylated antibody, the enzyme-labeled antibody and a serum containing the native antigen, reaction results between the native antigen and the antibodies, without competition or steric hindrance, to form a soluble sandwich complex. The interaction is illustrated by the following equation:

$$^{Enz}Ab_{(M)} + Ag_{C\text{-}Pep} + {}^{Btn}Ab_{(M)} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} {}^{Enz}Ab_{(M)} - Ag_{C\text{-}Pep} - {}^{Btn}Ab_{(M)}$$

$^{Btn}Ab_{(M)}$ = Biotinylated Monoclonal Ab (Excess Quantity)
$Ag_{C\text{-}Pep}$ = Native Antigen (Variable Quantity)
$^{Enz}Ab_{(M)}$ = Enzyme labeled Monoclonal Ab (Excess Quantity)
$^{Enz}Ab_{(M)} - Ag_{C\text{-}Pep} - {}^{Btn}Ab_{(M)}$ = Antigen-Antibodies complex
k_a = Rate Constant of Association
k_{-a} = Rate Constant of Dissociation

Simultaneously, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antibody. This interaction is illustrated below:
$^{Enz}Ab_{(M)} - Ag_{C\text{-}Pep} - {}^{Btn}Ab_{(M)}$
+ Streptavidin $_{C.W.} \Rightarrow$ Immobilize complex
Streptavidin $_{C.W.}$ = Streptavidin immobilized on well
Immobilized complex = sandwich complex bound to the solid surface

After equilibrium is attained, the antibody-bound fraction is separated from unbound antigen by decantation or aspiration. The enzyme activity in the antibody-bound fraction is directly proportional to the native antigen concentration. By utilizing several different serum references of known antigen values, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

4.0 REAGENTS AND MATERIALS PROVIDED

Materials Provided:

A. C-Peptide Calibrators – 2 ml/vial (Lyophilized) - Icons A-F
Six (6) vials of references for C-Peptide antigen at levels of 0(**A**), 0.2(**B**), 1.0(**C**), 2.0(**D**), 5.0(**E**), and 10.0(**F**) ng/ml. Reconstitute each vial with 2.0ml of distilled or deionized water. The reconstituted calibrators should be assayed immediately and can be stored for 8 hours at 2-8°C, then discarded. In order to store for a longer period of time, aliquot the reconstituted calibrators in cryo vials and store at -20°C for up to 30 days. Single use only. A preservative has been added.
Note: The calibrators, human serum based, were calibrated using a reference preparation, which was assayed against the WHO 1st IRR 84/510.

B. C-Peptide Enzyme Reagent – 13ml/vial - Icon Ⓔ
One (1) vial containing enzyme labeled affinity purified monoclonal mouse antibody, biotinylated monoclonal mouse IgG in buffer, dye, and preservative. Store at 2-8°C.

C. Streptavidin Plate – 96 wells – Icon ⇓
One 96-well microplate coated with streptavidin and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

D. Wash Solution Concentrate – 20 ml/vial - Icon
One (1) vial containing a surfactant in buffered saline. A preservative has been added. Store at 2-8°C.

E. Substrate A – 7.0ml/vial – Icon S^A
One (1) vial containing tetramethylbenzidine (TMB) in buffer. Store at 2-8°C.

F. Substrate B – 7.0ml/vial – Icon S^B
One (1) vial containing hydrogen peroxide (H_2O_2) in buffer. Store at 2-8°C.

G. Stop Solution – 8.0ml/vial - Icon STOP
One (1) vial containing a strong acid (1N HCl). Store at 2-8°C.

H. Product Instructions.

Note 1: Do not use reagents beyond the kit expiration date.
Note 2: Avoid extended exposure to heat and light. **Opened reagents are stable for sixty (60) days when stored at 2-8°C. Kit and component stability are identified on label.**
Note 3: Above reagents are for a single 96-well microplate

4.1 Required But Not Provided:
1. Pipette(s) capable of delivering 0.050ml (50µl) volumes with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100ml (100µl) and 0.350ml (350µl) volumes with a precision of better than 1.5%
3. Microplate washer or a squeeze bottle (optional).
4. Microplate Reader with 450nm and 620nm wavelength absorbance capability.
5. Absorbent Paper for blotting the microplate wells.
6. Plastic wrap or microplate cover for incubation steps.
7. Vacuum aspirator (optional) for wash steps.
8. Timer.
9. Storage container for storage of wash buffer.
10. Distilled or deionized water.
11. Quality Control Materials.

5.0 PRECAUTIONS

For In Vitro Diagnostic Use
Not for Internal or External Use in Humans or Animals

All products that contain human serum have been found to be non-reactive for Hepatitis B Surface antigen, HIV 1&2 and HCV antibodies by FDA required tests. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Edition, 1988, HHS.

Safe Disposal of kit components must be according to local regulatory and statutory requirement.

6.0 SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood serum in type, and the usual precautions in the collection of venipuncture samples should be observed. For accurate comparison to established normal values, a fasting morning serum sample should be obtained. The blood should be collected in a plain red-top venipuncture tube without additives. Allow the blood to clot. Centrifuge the specimen to separate the serum from the cells.

In patients receiving therapy with high biotin doses (i.e. >5mg/day), no sample should be taken until at least 8 hours after the last biotin administration, preferably overnight to ensure fasting sample.

Samples may be refrigerated at 2-8°C for a maximum period of two (2) days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20°C for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.100ml (100µl) of the specimen is required.

7.0 QUALITY CONTROL

Each laboratory should assay controls at levels in the low, normal and elevated range for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

8.0 REAGENT PREPARATION

3. **Wash Buffer**
Dilute contents of wash solution to 1000ml with distilled or deionized water in a suitable storage container. Store diluted buffer at 2-30°C for up to 60 days.
4. **Working Substrate Solution** – Stable for one year
Pour the contents of the amber vial labeled Solution 'A' into the clear vial labeled Solution 'B'. Place the yellow cap on the clear vial for easy identification. Mix and label accordingly. Store at 2 - 8°C.

Note 1: Do not use the working substrate if it looks blue.
Note 2: Do not use reagents that are contaminated or have bacteria growth.

9.0 TEST PROCEDURE

Before proceeding with the assay, bring all reagents, serum reference calibrators and controls to room temperature (20-27°C).
*****Test procedure should be performed by a skilled individual or trained professional*****

1. Format the microplates' wells for calibrator, control and patient specimen to be assayed in duplicate. **Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.**
2. Pipette 0.050 ml (50µl) of the appropriate calibrators, controls and samples into the assigned wells.
3. Add 0.100 ml (100µl) of the C-Peptide Enzyme Reagent to each well. **It is very important to dispense all reagents close to the bottom of the microwell.**
4. Swirl the microplate gently for 20-30 seconds to mix. Cover with a plastic wrap.
5. Incubate for 120 minutes at room temperature (20-25°C).
6. Discard the contents of the microplate by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper.
7. Add 0350ml (350µl) of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is used, fill each well to the top by squeezing the container. Avoiding air bubbles. Decant the wash and repeat two (2) additional times.**
8. Add 0.100 ml (100µl) of working substrate solution to all wells (see Reagent Preparation Section).

DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION

9. Incubate at room temperature for fifteen (15) minutes.
10. Add 0.050ml (50µl) of stop solution to each well and mix gently for 15-20 seconds. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) in a microplate reader. **The results should be read within thirty (30) minutes of adding the stop solution.**

NOTE: Always add reagents in the same order to minimize reaction time differences between wells.

10.0 CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of C-Peptide in unknown specimens.
1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the absorbance for each duplicate serum reference versus the corresponding C-Peptide concentration in ng/ml on linear graph paper (do not average the duplicates of the serum references before plotting).
3. Draw the best-fit curve through the plotted points.
4. To determine the concentration of C-Peptide for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in ng/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance (0.433) intersects the dose response curve at 1.03 ng/ml for the C-Peptide concentration (See Figure 1).

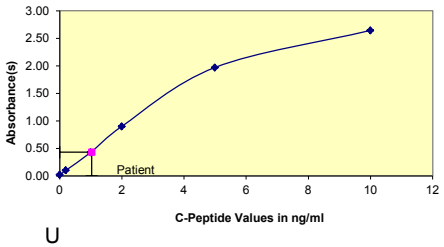
Note: Computer data reduction software designed for ELISA assays may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained.

*The data presented in Example 1 and Figure 1 is for illustration only and **should not** be used in lieu of a dose response curve prepared with each assay.

EXAMPLE 1

Sample I.D.	Well Number	Abs (A)	Mean Abs (B)	Value (ng/ml)
Cal A	A1	0.022	0.022	0
	B1	0.023		
Cal B	C1	0.097	0.103	0.2
	D1	0.107		
Cal C	E1	0.421	0.429	1
	F1	0.439		
Cal D	G1	0.889	0.901	2
	H1	0.910		
Cal E	A2	1.976	1.971	5
	B2	1.966		
Cal F	C2	2.717	2.643	10
	D2	2.570		
Ctrl 1	E2	0.429	0.433	1.03
	F2	0.437		
Ctrl 2	G2	1.861	1.887	4.64
	H2	1.913		
Patient 1	A3	0.388	0.405	0.82
	B3	0.421		

Figure 1



U

11.0 QC PARAMETERS

In order for the assay results to be considered valid the following criteria should be met:

1. The absorbance (OD) of calibrator 'A' should be ≤ 0.07
2. The absorbance (OD) of calibrator 'F' should be ≥ 1.3
3. Four out of six quality control pools should be within the established ranges.

12.0 RISK ANALYSIS

The MSDS and Risk Analysis Form for this product are available on request from Monobind Inc.

12.1 Assay Performance

1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. The addition of substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the substrate and stop solution should be added in the same sequence to eliminate any time-deviation during reaction.
6. Plate readers measure vertically. Do not touch the bottom of the wells.
7. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
8. Use components from the same lot. No intermixing of reagents from different batches.
9. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from Monobind IFU may yield inaccurate results.
10. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.
11. It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.
12. Risk Analysis- as required by CE Mark IVD Directive 98/79/EC - for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com.

12.2 Interpretation

1. ***Measurements and interpretation of results must be performed by a skilled individual or trained professional.***
2. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.
3. The reagents for the test system have been formulated to eliminate maximal interference; however, potential interaction between rare serum specimens and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for all kinds of immunoassays (Boscato LM, Stuart MC. 'Heterophilic antibodies: a problem for all immunoassays' Clin. Chem. 1988:3427-33). For diagnostic purposes, the results from this assay should be in combination with clinical examination, patient history and all other clinical findings.
4. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
5. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, Monobind shall have no liability.
6. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.

13.0 EXPECTED VALUES

C-Peptide values are consistently higher in plasma than in serum; Monobind advises that a serum sample be used for accurate determination. Compared with fasting values in non-obese non-diabetic individuals, C-Peptide levels are higher in obese non-diabetic subjects and lower in trained athletes.

Each laboratory is advised to establish its own ranges for normal and abnormal populations. These ranges are always dependent upon locale, population, laboratory, technique and specificity of the method.

It is important to keep in mind that any normal range establishment is dependent upon a multiplicity of factors like the specificity of the method, the locale, the population tested and the precision of the method in the hands of technicians. For these reasons each laboratory should depend upon the range of expected values established by the manufacturer only until an in-house range can be determined by the technicians using the method with a population indigenous to the area in which the laboratory is located.

Based on the clinical data gathered by Monobind in concordance with the published literature the following ranges have been assigned. **These ranges should be used as guidelines only:**

Adult (Normal)	**0.7 – 1.9 ng/ml**

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

The within and between assay precisions of the C-Peptide AccuBind® ELISA test system were determined by analyses on three different levels of pool control sera. The number (N), mean value(X), standard deviation (**σ**) and coefficient of variation (C.V.) for each of these control sera are presented in Table 1 and Table 2.

TABLE 1
Within Assay Precision (Values in ng/ml)

SAMPLE	N	X	σ	C.V.
Pool 1	20	1.43	0.11	7.7%
Pool 2	20	5.07	0.46	9.0%
Pool 3	20	7.81	0.73	9.3%

TABLE 2
Between Assay Precision* (Values in ng/ml)

SAMPLE	N	X	σ	C.V.
Pool 1	20	1.27	0.12	9.7%
Pool 2	20	5.40	0.54	9.9%
Pool 3	20	8.18	0.50	6.1%

*As measured in ten experiments in duplicate over ten days.

14.2 Sensitivity

The sensitivity (detection limit) was ascertained by determining the variability of the 0 ng/ml serum calibrator and using the 2σ (95% certainty) statistic to calculate the minimum dose. The assay sensitivity was found to be 0.020 ng/ml.

14.3 Accuracy

The C-Peptide AccuBind® ELISA test system was compared with a predicate radioimmunoassay assay. Biological specimens from population (symptomatic and asymptomatic) were used. (The values ranged from 0.2 ng/ml – 11.8ng/ml). The total number of such specimens was 124. The data obtained is displayed in Table 4 (see next column).

TABLE 4

Method	Mean (x)	Least Square Regression Analysis	Correlation Coefficient
Monobind (y)	1.068	y = 0.2079 + 0.8036(x)	0.962
Reference (x)	1.066		

Only slight amounts of bias between the C-Peptide AccuBind® ELISA test system and the reference method are indicated by the closeness of the mean values. The least square regression equation and correlation coefficient indicates excellent method agreement.

14.4 Specificity

The cross-reactivity of the C-Peptide AccuBind® ELISA test system to selected substances was evaluated by adding the interfering substance(s) to a serum matrix at the following concentration(s). The cross-reactivity was calculated by deriving a ratio between dose of interfering substance to dose of C-Peptide needed to produce the same absorbance.

Substance	Cross Reactivity	Concentration
C-Peptide	1.000	-
Proinsulin	0.120	100 ng/ml
Insulin	non-detectable	1.0 mIU/ml
Glucagon	non-detectable	150 ng/ml

15.0 REFERENCES

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Revision: 4 Date: 2019-Jul-16 DCO: 1353
MP2725 Product Code: 2725-300

Size		96(A)	192(B)
Reagent (fill)	A)	2ml set	2ml set
	B)	1 (13ml)	2 (13ml)
	C)	1 plate	2 plates
	D)	1 (20ml)	1 (20ml)
	E)	1 (7ml)	2 (7ml)
	F)	1 (7ml)	2 (7ml)
	G)	1 (8ml)	2 (8ml)

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IVD 2°-8° CE
EC REP CEpartner4U, Esdoornlaan 13 3951 DBMaarn, The Neatherlands www.cepartner4u.eu

Please visit our website to learn more about our products and services.

Glossary of Symbols
(EN 980/ISO 15223)

IVD — In Vitro - Diagnostic Medical Device

2°-8° — Temperature Limitation Storage Condition (2-8°C)

Consult Instructions for Use

REF — Catalogue Number

Σ — Contains Sufficient Test for Σ

LOT — Batch Code

Used By (Expiration Day)

Date of Manufacturer

Manufacturer

EC REP — Authorized Rep in European Country

CE — European Conformity

Instructions for Use

TM-CYFRA 21-1 ELISA

IVD

CE

REF **EIA-5070**

Σ **96**

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Please use only the valid version of the Instructions for Use provided with the kit.
Verwenden Sie nur die jeweils gültige, im Testkit enthaltene, Gebrauchsanweisung.
Si prega di usare la versione valida delle istruzioni per l'uso a disposizione con il kit.
Por favor, se usa solo la version valida de la metodico técnico incluido aqui en el kit.
Utilisez seulement la version valide des Instructions d'utilisation fournies avec le kit.

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1 INTRODUCTION

1.1 Intended Use

The **DRG TM-CYFRA 21-1 ELISA** is an enzyme immunoassay for the quantitative *in vitro diagnostic* measurement of CYFRA 21-1 in serum or plasma (heparin- or citrate plasma).

1.2 Summary and Explanation

Cytokeratins are epithelial markers whose expression is not lost during malignant transformation. CYFRA 21-1 is a cytokeratin-19 fragment that is soluble in serum and can be used as circulating tumor marker. Although expressed in all body tissues, its major occurrence is in the lung, particularly in lung cancer tissues. CYFRA 21-1 is a sensitive and specific tumor marker of non-small-cell lung cancer (NSCLC), especially of squamous cell subtype (1,2,3). It also reflects the extent of the disease and has an independent prognostic role along with performance status and disease stage in NSCLC (4,5,6). In addition, detection of serum CYFRA 21-1 allows for identification of high risk patients that may benefit from adjuvant chemotherapy (7), and enables the early detection of progressive disease in recurrent NSCLC (8). Additionally, CYFRA 21-1 has been described as a useful marker for esophageal squamous cell carcinoma (9) and for therapy monitoring of bladder cancer (10).

The TM-CYFRA 21-1 ELISA uses the two mouse monoclonal antibodies KS19.1 and BM19.21 to determine cytokeratin-19 fragments.

2 PRINCIPLE OF THE TEST

The DRG TM-CYFRA 21-1 ELISA is a solid phase enzyme-linked immunosorbent assay (ELISA) based on the **sandwich principle.**

The microtiter wells are coated with a monoclonal (mouse) antibody directed towards a unique antigenic site of the CYFRA 21-1 molecule.

An aliquot of patient sample containing endogenous CYFRA 21-1 is incubated in the coated well with enzyme conjugate, which is an anti-CYFRA 21-1 antibody conjugated with horseradish peroxidase. After incubation the unbound conjugate is washed off.

The amount of bound peroxidase conjugate is proportional to the concentration of CYFRA 21-1 in the sample.

Having added the substrate solution, the intensity of colour developed is proportional to the concentration of CYFRA 21-1 in the patient sample.

3 WARNINGS AND PRECAUTIONS

1. This kit is for in vitro diagnostic use only. For professional use only.
2. All reagents of this test kit which contain human serum or plasma have been tested and confirmed negative for HIV I/II, HBsAg and HCV by FDA approved procedures. All reagents, however, should be treated as potential biohazards in use and for disposal.
3. Before starting the assay, read the instructions completely and carefully. Use the valid version of instructions for use provided with the kit. Be sure that everything is understood.
4. The microplate contains snap-off strips. Unused wells must be stored at 2 °C to 8 °C in the sealed foil pouch and used in the frame provided.
5. Pipetting of samples and reagents must be done as quickly as possible and in the same sequence for each step.
6. Use reservoirs only for single reagents. This especially applies to the substrate reservoirs. Using a reservoir for dispensing a substrate solution that had previously been used for the conjugate solution may turn solution colored. Do not pour reagents back into vials as reagent contamination may occur.
7. Mix the contents of the microplate wells thoroughly to ensure good test results. Do not reuse microwells.
8. Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
9. Allow the reagents to reach room temperature (21 °C to 26 °C) before starting the test. Temperature will affect the absorbance readings of the assay. However, values for the patient samples will not be affected.
10. Never pipet by mouth and avoid contact of reagents and specimens with skin and mucous membranes.
11. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.
12. Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of reagents or specimens may give false results.
13. Handling should be done in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.
14. Do not use reagents beyond expiry date as shown on the kit labels.
15. All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microtiter plate readers.
16. Do not mix or use components from kits with different lot numbers. It is advised not to exchange wells of different plates even of the same lot. The kits may have been shipped or stored under different conditions and the binding characteristics of the plates may result slightly different.
17. Avoid contact with Stop Solution containing 0.5 M H_2SO_4. It may cause skin irritation and burns.
18. Some reagents contain Proclin 300, BND and/or MIT as preservatives. In case of contact with eyes or skin, flush immediately with water.
19. TMB substrate has an irritant effect on skin and mucosa. In case of possible contact, wash eyes with an abundant volume of water and skin with soap and abundant water. Wash contaminated objects before reusing them. If inhaled, take the person to open air.
20. Chemicals and prepared or used reagents have to be treated as hazardous waste according to the national biohazard safety guideline or regulation.
21. For information on hazardous substances included in the kit please refer to Safety Data Sheets.
    Safety Data Sheets for this product are available upon request directly from DRG.

4 REAGENTS

4.1 Reagents provided

1. ***Microtiterwells***, 12 x 8 (break apart) strips, 96 wells;
   Wells coated with anti-CYFRA 21-1 antibody (monoclonal).
2. ***Standard (Standard 0 - 4)***, 5 vials, 1 mL each, lyophilized;
   Concentrations: 0 - 3 - 10 - 25 - 50 ng/mL
   See “Reagent Preparation“.
   Contain non-mercury preservative.
3. ***Control Low & High***, 2 vials, 1 mL each, lyophilized;
   For control values and ranges please refer to vial label or QC-Datasheet.
   See “Reagent Preparation“.
   Contain non-mercury preservative.
4. ***Sample Diluent***, 1 vial, 3 mL, ready to use;
   Contains non-mercury preservative.
5. ***Assay Buffer***, 1 vial, 7 mL, ready to use;
   Contains non-mercury preservative.
6. ***Enzyme Conjugate***, 1 vial, 1.2 mL, ready to use,
   Anti-CYFRA 21-1 antibody conjugated with horseradish peroxidase;
   Contains non-mercury preservative.
7. ***Substrate Solution***, 1 vial, 14 mL, ready to use;
   Tetramethylbenzidine (TMB).
8. ***Stop Solution***, 1 vial, 14 mL, ready to use;
   Contains 0.5 M H_2SO_4,
   Avoid contact with the stop solution. It may cause skin irritations and burns.
9. ***Wash Solution***, 1 vial, 30 mL (40X concentrated);
   See “Reagent Preparation“.

Note: Additional *Sample Diluent* for sample dilution is available upon request.

4.2 Materials required but not provided

- A microtiter plate calibrated reader (450 nm ± 10 nm) (e.g. the DRG Instruments Microtiter Plate Reader)
- Calibrated variable precision micropipettes
- Absorbent paper
- Distilled or deionized water
- Timer
- Graph paper or software for data reduction

4.3 Storage Conditions

When stored at 2 °C to 8 °C unopened reagents will retain reactivity until expiration date. Do not use reagents beyond this date.

Opened reagents must be stored at 2 °C to 8 °C. Microtiter wells must be stored at 2 °C to 8 °C. Once the foil bag has been opened, care should be taken to close it tightly again.
Opened kits retain activity for 8 weeks if stored as described above.

4.4 Reagent Preparation

Bring all reagents and required number of strips to room temperature prior to use.

Standards

Reconstitute the lyophilized contents of each vial with 1 mL deionized water and let stand for at least 10 minutes at room temperature. Mix several times before use.

Note: *The reconstituted standards are stable for 8 weeks at 2 °C to 8 °C.*
For longer storage freeze at -20 °C.

Controls

Reconstitute the lyophilized content each vial with 1 mL deionized water and let stand for at least 10 minutes at room temperature. Mix the control several times before use.

Note: *The reconstituted controls are stable for 8 weeks at 2 °C to 8 °C.*
For longer storage freeze at -20 °C.

Wash Solution

Add deionized water to the 40X concentrated Wash Solution.
Dilute 30 mL of concentrated *Wash Solution* with 1170 mL deionized water to a final volume of 1200 mL.
The diluted Wash Solution is stable for 2 weeks at room temperature.

4.5 Disposal of the Kit

The disposal of the kit must be made according to the national regulations. Special information for this product is given in the Safety Data Sheet, section 13.

4.6 Damaged Test Kits

In case of any severe damage to the test kit or components, DRG has to be informed in writing, at the latest, one week after receiving the kit. Severely damaged single components should not be used for a test run. They have to be stored until a final solution has been found. After this, they should be disposed according to the official regulations.

5 SPECIMEN COLLECTION AND PREPARATION

Serum or plasma (heparin- or citrate plasma) can be used in this assay.
The use of EDTA plasma results in increased values.

Do not use haemolytic, icteric or lipaemic specimens.
Please note: Samples containing sodium azide should not be used in the assay.

5.1 Specimen Collection

Serum:
Collect blood by venipuncture (e.g. Sarstedt Monovette for serum), allow to clot, and separate serum by centrifugation at room temperature. Do not centrifuge before complete clotting has occurred. Patients receiving anticoagulant therapy may require increased clotting time.

Plasma:
Whole blood should be collected into centrifuge tubes containing anti-coagulant (e.g. Sarstedt Monovette with the appropriate plasma preparation) and centrifuged immediately after collection.

5.2 Specimen Storage and Preparation

Specimens should be capped and may be stored for up to 5 days at 2 °C to 8 °C prior to assaying.
Specimens held for a longer time (up to 18 months) should be frozen only once at -20 °C prior to assay. Thawed samples should be inverted several times prior to testing.

5.3 Specimen Dilution

If in an initial assay, a specimen is found to contain more than the highest standard, the specimens can be diluted with *Sample Diluent* and re-assayed as described in Assay Procedure.
For the calculation of the concentrations this dilution factor has to be taken into account.

Example:
a) dilution 1:10: 10 µL sample + 90 µL *Sample Diluent* (mix thoroughly)
b) dilution 1:100: 10 µL dilution a) 1:10 + 90 µL *Sample Diluent* (mix thoroughly).

6 ASSAY PROCEDURE

6.1 General Remarks

- All reagents and specimens must be allowed to come to room temperature before use. All reagents must be mixed without foaming.
- Once the test has been started, all steps should be completed without interruption.
- Use new disposal plastic pipette tips for each standard, control or sample in order to avoid cross contamination.
- Absorbance is a function of the incubation time and temperature. Before starting the assay, it is recommended that all reagents are ready, caps removed, all needed wells secured in holder, etc. This will ensure equal elapsed time for each pipetting step without interruption.
- As a general rule the enzymatic reaction is linearly proportional to time and temperature.

6.2 Test Procedure

Each run must include a standard curve.

1. Secure the desired number of Microtiter wells in the frame holder.
2. Dispense **50 µL *Assay Buffer*** into each well.
3. Dispense **10 µL *Enzyme Conjugate*** into each well.
4. Dispense **50 µL** of each ***Standard, Control*** and **samples** with new disposable tips into appropriate wells. Thoroughly mix for 10 seconds. It is important to have a complete mixing in this step.
5. Incubate for **60 minutes** at room temperature.
6. Briskly shake out the contents of the wells.
   Rinse the wells **3 times** with **350 µL** diluted Wash Solution per well. Strike the wells sharply on absorbent paper to remove residual droplets.
   Important note:
   The sensitivity and precision of this assay is markedly influenced by the correct performance of the washing procedure!
7. Add **100 µL** of ***Substrate Solution*** to each well.
8. Incubate for **15 minutes** at room temperature.
9. Stop the enzymatic reaction by adding **100 µL** of ***Stop Solution*** to each well.
10. Determine the absorbance (OD) of each well at **450 nm ± 10 nm** with a microtiter plate reader.
    It is recommended that the wells be read **within 10 minutes** after adding the *Stop Solution*.

6.3 Calculation of Results

1. Calculate the average absorbance values for each set of standards, controls and patient samples.
2. Using linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.
4. Automated method: The results in the Instructions for Use have been calculated automatically using a 4-Parameter curve fit. (4 Parameter Rodbard or 4 Parameter Marquardt are the preferred methods.) Other data reduction functions may give slightly different results.
5. The concentration of the samples can be read directly from this standard curve. Samples with concentrations higher than that of the highest standard have to be further diluted or reported as > 50 ng/mL. For the calculation of the concentrations this dilution factor has to be taken into account.

6.3.1 Example of Typical Standard Curve

The following data is for demonstration only and **cannot** be used in place of data generations at the time of assay.

Standard	Optical Units (450 nm)
Standard 0 (0 ng/mL)	0.05
Standard 1 (3 ng/mL)	0.23
Standard 2 (10 ng/mL)	0.63
Standard 3 (25 ng/mL)	1.37
Standard 4 (50 ng/mL)	2.35

7 EXPECTED NORMAL VALUES

It is strongly recommended that each laboratory should determine its own normal and abnormal values.

In a study conducted with apparently healthy adults, using the DRG TM-CYFRA 21-1 ELISA the following data were observed:

Population	n	Mean (ng/mL)	Median (ng/mL)	5^{th} - 95^{th} Percentile (ng/mL)	Range (min. - max.) (ng/mL)
Males	121	0.61	0.60	0.10 - 1.33	0.00 - 2.53
Females	119	0.58	0.46	0.00 - 1.38	0.00 - 1.98

Several studies recommended a cut-off concentration of 3.3 ng/mL for CYFRA 21-1, since all patients without disease and 95% of patients with benign lung diseases are found below this value (1,2,3).

The results alone should not be the only reason for any therapeutic consequences. The results should be correlated to other clinical observations and diagnostic tests.

8 QUALITY CONTROL

Good laboratory practice requires that controls be run with each calibration curve. A statistically significant number of controls should be assayed to establish mean values and acceptable ranges to assure proper performance.

It is recommended to use control samples according to state and federal regulations. The use of control samples is advised to assure the day to day validity of results. Use controls at both normal and pathological levels.

The controls and the corresponding results of the QC-Laboratory are stated in the QC certificate added to the kit. The values and ranges stated on the QC sheet always refer to the current kit lot and should be used for direct comparison of the results.

It is also recommended to make use of national or international Quality Assessment programs in order to ensure the accuracy of the results.

Employ appropriate statistical methods for analysing control values and trends. If the results of the assay do not fit to the established acceptable ranges of control materials patient results should be considered invalid.

In this case, please check the following technical areas: Pipetting and timing devices; photometer, expiration dates of reagents, storage and incubation conditions, aspiration and washing methods.

After checking the above mentioned items without finding any error contact your distributor or DRG directly.

9 PERFORMANCE CHARACTERISTICS

9.1 Assay Dynamic Range

The range of the assay is between 0.079 ng/mL - 50 ng/mL.

9.2 Specificity of Antibodies (Cross Reactivity)

The antibodies used for the DRG TM-CYFRA 21-1 ELISA are specific for Keratin 19.

9.3 Sensitivity

The Limit of Blank (LoB) is 0.079 ng/mL.
The Limit of Detection (LoD) is 0.185 ng/mL.
The Limit of Quantification (LoQ) is 0.343 ng/mL.

9.4 Reproducibility

9.4.1 Intra Assay

The within assay variability is shown below:

Sample	n	Mean (ng/mL)	CV (%)
1	80	1.90	6.4
2	80	4.31	3.5
3	80	12.68	2.7
4	80	33.89	3.4

9.4.2 Inter Assay

The between assay variability is shown below:

Sample	n	Mean (ng/mL)	CV (%)
1	80	1.90	11.7
2	80	4.31	7.1
3	80	12.68	5.5
4	80	33.89	5.7

9.4.3 Inter-Lot

The inter-assay (between-lots) variation was determined by repeated measurements of samples with 3 different kit lots.

Sample	n	Mean (ng/mL)	CV (%)
1	18	2.62	2.5
2	18	8.68	5.1
3	18	11.07	2.5
4	18	32.75	5.2

9.5 Recovery

Samples have been spiked by adding CYFRA 21-1 solutions with known concentrations in a 1:1 ratio.
The % recovery has been calculated by multiplication of the ratio of the measurements and the expected values with 100 (expected value = (endogenous CYFRA 21-1 + added CYFRA 21-1)/2; because of a 1:2 dilution of serum with spike material).

		Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
Concentration (ng/mL)		2.24	5.90	10.06	15.98	23.14	38.67
Average Recovery (%)		91.7	94.5	95.4	94.1	94.9	94.8
Range of Recovery (%)	from	87.7	89.1	89.3	91.4	91.3	90.9
	to	101.8	105.5	104.1	98.6	100.1	98.1

9.6 Linearity

		Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
Concentration (ng/mL)		11.60	26.95	45.05	15.55	22.52	37.62
Average Recovery (%)		99.8	103.8	101.8	103.8	104.3	100.1
Range of Recovery (%)	from	95.5	100.8	95.9	101.8	99.8	95.8
	to	104.0	108.3	109.1	108.7	107.2	104.6

10 LIMITATIONS OF USE

Reliable and reproducible results will be obtained when the assay procedure is performed with a complete understanding of the package insert instruction and with adherence to good laboratory practice.

Any improper handling of samples or modification of this test might influence the results.

10.1 Interfering Substances

Haemoglobin (up to 4 mg/mL), Bilirubin (up to 0.5 mg/mL) and Triglyceride (up to 7.5 mg/mL) have no influence on the assay results.

The assay contains reagents to minimize interference of HAMA and heterophilic antibodies.

10.2 Drug Interferences

Until today no substances (drugs) are known to us, which have an influence to the measurement of CYFRA 21-1 in a sample.

10.3 High-Dose-Hook Effect

Hook effect was not observed in this test up to a concentration of 1000 ng/mL of CYFRA 21-1.

11 LEGAL ASPECTS

11.1 Reliability of Results

The test must be performed exactly as per the manufacturer's instructions for use. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable national standards and/or laws. This is especially relevant for the use of control reagents. It is important to always include, within the test procedure, a sufficient number of controls for validating the accuracy and precision of the test.

The test results are valid only if all controls are within the specified ranges and if all other test parameters are also within the given assay specifications. In case of any doubt or concern please contact DRG.

11.2 Therapeutic Consequences

Therapeutic consequences should never be based on laboratory results alone even if all test results are in agreement with the items as stated under point 11.1. Any laboratory result is only a part of the total clinical picture of a patient.

Only in cases where the laboratory results are in acceptable agreement with the overall clinical picture of the patient should therapeutic consequences be derived.

The test result itself should never be the sole determinant for deriving any therapeutic consequences.

11.3 Liability

Any modification of the test kit and/or exchange or mixture of any components of different lots from one test kit to another could negatively affect the intended results and validity of the overall test. Such modification and/or exchanges invalidate any claim for replacement.

Claims submitted due to customer misinterpretation of laboratory results subject to point 11.2 are also invalid.
Regardless, in the event of any claim, the manufacturer's liability is not to exceed the value of the test kit. Any damage caused to the test kit during transportation is not subject to the liability of the manufacturer.

1 EINLEITUNG

Der **DRG TM-CYFRA 21-1 ELISA** wird zur quantitativen Bestimmung von CYFRA 21-1 in Serum oder Plasma (Heparin- oder Zitratplasma) eingesetzt.

Nur für In-vitro Diagnostik.

1.1 Zusammenfassung und Erklärung

Zytokeratine sind epitheliale Marker, deren Expression im Zuge maligner Transformationen nicht verloren geht. CYFRA 21-1 ist ein Fragment des Cytokeratin-19, das in gelöster Form im Serum vorliegt und deshalb als zirkulierender Tumormarker verwendet werden kann. Prinzipiell wird CYFRA 21-1 in allen Geweben exprimiert, vor allem jedoch in Tumorgewebe der Lunge. Deshalb ist CYFRA 21-1 ein sensitiver und spezifischer Tumormarker des nicht-kleinzelligen Lungenkarzinoms (NSCLC), vor allem des squamösen Subtyps (1,2,3). Es spiegelt auch das Ausmaß der Krankheit wieder, gilt als unabhängiger prognostischer Faktor und reflektiert das Tumorstaging bei NSCLC (4,5,6). Weiterhin ermöglicht CYFRA 21-1 die Identifizierung von Hochrisiko-Patienten, die von einer adjuvanten Chemotherapie profitieren könnten (7) und erlaubt die Detektion eines fortschreitenden Rezidivs bei NSCLC (8). Ferner wurde CYFRA 21-1 als Marker für das squamöse Ösophaguskarzinom beschrieben (9) sowie als Marker zum Therapiemonitoring bei Blasentumoren (10).

Zur Bestimmung der Zytokeratin-19 Fragmente werden im CYFRA 21-1 ELISA die zwei monoklonalen Antikörper KS19.1 und BM19.21 verwendet.

2 TESTPRINZIP

Der DRG **TM-CYFRA 21-1** ELISA ist ein Festphasen-Enzymimmunoassay, der auf der **Sandwichtechnik** basiert.

Die Wells der Mikrotiterplatten sind mit einem monoklonalen Antikörper beschichtet, der gegen eine definierte Antikörper-Bindungsstelle des CYFRA 21-1-Moleküls gerichtet ist.

Die Proben werden in die beschichteten Wells gegeben und mit einem Enzymkonjugat inkubiert. Das Konjugat enthält einen anti-CYFRA 21-1-Antikörper, der mit Meerrettichperoxidase konjugiert ist. Es wird ein Sandwichkomplex gebildet.

Das nicht gebundene Konjugat wird durch Waschen der Wells entfernt. Anschließend wird die Substratlösung zugegeben und die Farbentwicklung nach einer definierten Zeit gestoppt. Die Intensität der gebildeten Farbe ist proportional der CYFRA 21-1-Konzentration in der Probe. Die Extinktion wird bei 450 nm mit einem Mikrotiterplattenleser gemessen.

3 VORSICHTSMAßNAHMEN

- Dieser Kit ist nur zum in vitro diagnostischen Gebrauch geeignet.
- Nur die gültige, im Testkit enthaltene, Gebrauchsanweisung verwenden.
- Informationen zu im Kit enthaltenen gefährlichen Substanzen entnehmen Sie bitte dem Sicherheitsdatenblatt.
- Alle Bestandteile dieses Testkits, die humanes Serum oder Plasma enthalten, wurden mit FDA-geprüften Methoden auf HIV I/II, HbsAg und HCV getestet und als negativ bestätigt. Jedoch sollten alle Bestandteile im Umgang und bei der Entsorgung wie mögliche Gefahrenstoffe betrachtet werden.
- Der Kontakt mit der *Stop Solution* sollte vermieden werden, da sie 0,5 M H_2SO_4 enthält. Schwefelsäure kann Hautreizungen und Verbrennungen verursachen.
- Nicht mit dem Mund pipettieren und den Kontakt von Kitbestandteilen und Proben mit Haut und Schleimhäuten vermeiden.
- In den Bereichen, in denen Proben oder Kitbestandteile verwendet werden, nicht rauchen, essen oder Kosmetika verwenden.
- Beim Umgang mit Proben oder Reagenzien Einweg-Latexhandschuhe tragen. Die Verunreinigung von Reagenzien oder Proben mit Mikroben kann zu falschen Ergebnissen führen.
- Der Gebrauch sollte gemäß der Vorschriften einer entsprechenden nationalen Gefahrenstoff-Sicherheitsrichtlinie erfolgen.
- Reagenzien nicht nach dem auf dem Kit-Etikett angegebenen Verfallsdatum verwenden.
- Alle im Kit-Protokoll angegebenen Mengen müssen genau eingehalten werden. Optimale Ergebnisse können nur durch Verwendung kalibrierter Pipetten und Mikrotiterplatten-Lesegeräte erreicht werden.
- Komponenten von Kits mit unterschiedlichen Lotnummern nicht untereinander vertauschen. Es wird empfohlen, keine Wells von verschiedenen Platten zu verwenden, auch nicht, wenn es sich um das gleiche Lot handelt. Die Kits können unter anderen Bedingungen gelagert oder versendet worden sein, so dass die Bindungscharakteristik der Platten leicht unterschiedlich ausfällt.
- Chemikalien und zubereitete oder bereits benutzte Reagenzien müssen gemäß den nationalen Gefahrenstoffvorschriften wie gefährlicher Abfall behandelt werden.
- Sicherheitsdatenblätter für dieses Produkt sind auf Anfrage direkt von der Firma DRG Instruments GmbH erhältlich.

4 BESTANDTEILE DES KITS

4.1 Kitinhalt

1. ***Microtiterwells***, 96 Wells, 12 x 8 Wells (einzeln brechbar);
   Mit anti-CYFRA 21-1-Antikörper (monoklonal) beschichtet.
2. ***Standard (Standard 0 - 4)***, 5 Fläschchen, je 1 mL, lyophilisiert;
   Konzentrationen: 0 - 3 - 10 - 25 - 50 ng/mL
   Siehe „Vorbereitung der Reagenzien“.
   Enthält quecksilberfreies Konservierungsmittel.
3. ***Control Low & High*** (Kontrolle), 2 Fläschchen, je 1 mL, lyophilisiert
   Kontrollwerte und -bereiche entnehmen Sie bitte dem Fläschchenetikett oder dem QC-Datenblatt.
   Siehe „Vorbereitung der Reagenzien“.
   Enthält quecksilberfreies Konservierungsmittel.
4. ***Sample Diluent*** (Probenverdünnungsmedium), 1 Fläschchen, 3 mL, gebrauchsfertig;
   Enthält quecksilberfreies Konservierungsmittel.
5. ***Assay Buffer*** (Assaypuffer), 1 Fläschchen, 7 mL, gebrauchsfertig;
   Enthält quecksilberfreies Konservierungsmittel.
6. ***Enzyme Conjugate*** (Enzymkonjugat), 1 Fläschchen, 1,2 mL, gebrauchsfertig;
   Anti-CYFRA 21-1-Antikörper mit Meerrettichperoxidase konjugiert;
   Enthält quecksilberfreies Konservierungsmittel.
7. ***Substrate Solution*** (Substratlösung), 1 Fläschchen, 14 mL, gebrauchsfertig;
   Substratlösung TMB.
8. ***Stop Solution*** (Stopplösung), 1 Fläschchen, 14 mL, gebrauchsfertig;
   enthält 0,5 M H_2SO_4,
   Kontakt mit der Stopplösung vermeiden! Kann Hautreizungen und Verbrennungen verursachen.
9. ***Wash Solution*** (Waschlösung), 1 Fläschchen, 30 mL, **40X** konzentriert;
   Siehe „Vorbereitung der Reagenzien“.

Anmerkung: Zusätzliches *Sample Diluent* zur Probenverdünnung ist auf Anfrage erhältlich.

4.2 Erforderliche aber nicht enthaltene Geräte und Materialien

- Kalibriertes Mikrotiterplattenlesegerät mit 450 nm ± 10 nm Filter), (z.B. das DRG Instruments Mikrotiterplattenlesegerät)
- Kalibrierte variable Präzisions-Mikropipette
- Saugfähiges Papier
- Destilliertes Wasser
- Laborwecker
- Millimeterpapier oder Software zur Datenauswertung

4.3 Lagerung und Haltbarkeit des Kits

Die ungeöffneten Reagenzien behalten bei Lagerung um 2 °C bis 8 °C ihre Reaktivität bis zum Verfallsdatum. Nach dem Verfallsdatum die Reagenzien nicht mehr verwenden.

Nach dem Öffnen sollten alle Reagenzien bei 2 °C bis 8 °C gelagert werden.

Die Mikrotiterwells sollten bei 2 °C bis 8 °C gelagert werden. Der einmal geöffnete Folienbeutel sollte stets sehr sorgfältig wieder verschlossen werden.

Unter den beschriebenen Lagerbedingungen behalten geöffnete Kits 8 Wochen ihre Reaktivität.

4.4 Vorbereitung der Reagenzien

Alle Reagenzien sowie die benötigte Anzahl von Wells sollen vor dem Gebrauch auf Raumtemperatur gebracht werden.

Standards

Das Lyophilisat in jedem Fläschchen mit 1 mL destilliertem Wasser auflösen und mindestens 10 Minuten bei Raumtemperatur stehen lassen. Vor der Verwendung mehrere Male mischen.

Achtung: *Bei 2 °C bis 8 °C sind die rekonstituierten Standards 8 Wochen haltbar.*
Für eine längere Aufbewahrung bei -20 °C einfrieren.

Control

Das Lyophilisat in jedem Fläschchen mit 1 mL destilliertem Wasser auflösen und mindestens 10 Minuten bei Raumtemperatur stehen lassen. Vor der Verwendung mehrere Male mischen.

Achtung: *Bei 2 °C bis 8 °C sind die rekonstituierten Kontrollen 8 Wochen haltbar.*
Für eine längere Aufbewahrung bei -20 °C einfrieren.

Wash Solution

Die 40-fach konzentrierte *Wash Solution* (30 mL) mit 1170 mL destilliertem Wasser auf ein Gesamtvolumen von 1200 mL verdünnen.

Die verdünnte Waschlösung ist bei Raumtemperatur für 2 Wochen stabil.

4.5 Entsorgung des Kits

Die Entsorgung des Kits muss gemäß den nationalen gesetzlichen Vorschriften erfolgen. Spezielle Informationen für dieses Produkt finden Sie im Sicherheitsdatenblatt, Abschnitt 13.

4.6 Beschädigte Testkits

Im Falle einer starken Beschädigung des Testkits oder der Komponenten muss die Firma DRG in schriftlicher Form spätestens eine Woche nach Erhalt des Kits informiert werden. Stark beschädigte Einzelkomponenten sollten nicht für den Testlauf verwendet werden. Sie müssen gelagert werden bis eine endgültige Lösung gefunden wurde. Danach sollten Sie gemäß den offiziellen Richtlinien entsorgt werden.

5 PROBENVORBEREITUNG

Serum oder Plasma (Heparin- oder Zitratplasma) kann in diesem Test als Probenmaterial eingesetzt werden.
EDTA-Plasma führt zu erhöhten Werten.

Lipämische, ikterische und/oder stark hämolysierte Proben sollten nicht verwendet werden.
Achtung: Proben, die Natriumazid enthalten, sollten nicht verwendet werden.

5.1 Probenentnahme

Serum:
Blut durch Venenpunktion entnehmen (z.B. mit Sarstedt Monovette für Serum), gerinnen lassen und das Serum durch Zentrifugation bei Raumtemperatur abtrennen. Vor der Zentrifugation muss die Gerinnung vollständig abgeschlossen sein. Bei Patienten, die Antikoagulantien erhalten, kann die Gerinnungszeit länger dauern.

Plasma:
Die Blutentnahme erfolgt mit Röhrchen, die ein Antikoagulanz enthalten (z.B.: Sarstedt Monovette – mit entsprechender Plasma-Präparierung). Das Plasma wird als Überstand nach einer Zentrifugation gewonnen.

5.2 Probenaufbewahrung

Proben sollten stets gut verschlossen sein und können vor Testbeginn bis zu 5 Tage bei 2 °C bis 8 °C gelagert werden.
Für eine längere Aufbewahrung (bis zu 18 Monate) sollten die Proben eingefroren bei -20 °C bis zum Testbeginn gelagert werden. Nur einmal einfrieren. Aufgetaute Proben sollten vor Testbeginn vorsichtig durchmischt werden, ohne Schaumbildung.

5.3 Probenverdünnung

Wenn in einem ersten Testdurchlauf bei einer Probe eine Konzentration höher als der höchste Standard gefunden wird, kann diese Probe mit *Sample Diluent* weiter verdünnt und nochmals bestimmt werden. Die Verdünnung muss jedoch bei der Berechnung der Konzentration beachtet werden.

Beispiel:
a) Verdünnung 1:10: 10 µL Probe + 90 µL *Sample Diluent* gründlich mischen)
b) Verdünnung 1:100: 10 µL Verdünnung a) 1:10 + 90 µL *Sample Diluent* (gründlich mischen).

6 TESTDURCHFÜHRUNG

6.1 Allgemeine Hinweise

- Alle Reagenzien und Proben müssen vor Gebrauch auf Raumtemperatur gebracht und gut durchmischt werden. Dabei sollte Schaumbildung vermieden werden.
- Wenn die Testdurchführung einmal begonnen wurde, muss sie ohne Unterbrechung zu Ende geführt werden.
- Für jeden Standard, jede Kontrolle oder Probe eine neue Plastikspitze verwenden, um Verschleppungen zu vermeiden.
- Die Optische Dichte ist abhängig von Inkubationszeit und Temperatur. Deshalb ist es notwendig, vor Beginn der Testdurchführung alle Reagenzien in einen arbeitsbereiten Zustand zu bringen, die Deckel der Fläschchen zu öffnen, alle benötigten Wells in den Halter zu setzen. Nur eine solche Vorbereitung garantiert gleiche Zeiten für jeden Pipettiervorgang ohne Pausen.
- Als generelle Regel gilt, dass die enzymatische Reaktion linear proportional zu Zeit und Temperatur ist.

6.2 Testdurchführung

Jeder Lauf muss eine Standardkurve beinhalten.

1. Die benötigte Anzahl Wells in der Halterung befestigen.
2. **50 µL *Assay Buffer*** in jedes Well geben.
3. **10 µL *Enzyme Conjugate*** in jedes Well geben.
4. **Je 50 µL *Standard, Control*** und **Proben** mit neuen Plastikspitzen in die entsprechenden Wells geben
   Für 10 Sekunden gut schütteln. Es ist sehr wichtig, in diesem Schritt eine komplette Durchmischung zu erreichen.
5. **60 Minuten** bei Raumtemperatur inkubieren.
6. Den Inhalt der Wells kräftig ausschütteln. Wells **3-mal** mit **350 µL** verdünnter *Wash Solution* waschen. Verbleibende Flüssigkeit durch Ausklopfen der Wells auf saugfähigem Papier entfernen.
   Achtung: Die Sensitivität und Präzision dieses Assays wird erheblich beeinflusst von der korrekten Durchführung des Waschschrittes!
7. **100 µL *Substrate Solution*** in jedes Well geben.
8. **15 Minuten** bei Raumtemperatur inkubieren.
9. Die enzymatische Reaktion durch Zugabe von **100 µL *Stop Solution*** in jedes Well abstoppen.
10. Die Optische Dichte bei **450 nm ± 10 nm** mit einem Mikrotiterplatten-Lesegerät innerhalb von **10 Minuten** nach Zugabe der ***Stop Solution*** bestimmen.

6.3 Ergebnisermittlung

1. Die durchschnittlichen Werte der Optischen Dichte (OD) für jedes Set von Standards, Controls und Patientenproben bestimmen.
2. Eine Standardkurve ermitteln durch Auftragen der mittleren Optischen Dichte jedes Standards gegen die Konzentration, wobei der OD-Wert auf der vertikalen (Y) Achse und die Konzentration auf der horizontalen (X) Achse eingetragen wird.
3. Unter Verwendung der mittleren OD wird für jede Probe die entsprechende Konzentration aus der Standardkurve ermittelt.
4. Automatische Methode: Die in der Gebrauchsanweisung angegebenen Werte wurden automatisch mit Hilfe der 4 Parameter-Gleichung bestimmt. (4 Parameter Rodbard oder 4 Parameter Marquardt sind die bevorzugten Methoden.) Andere Auswertungsfunktionen können leicht abweichende Werte ergeben.
5. Die Konzentration der Proben kann direkt von der Standardkurve abgelesen werden. Proben, die eine höhere Konzentration als die des höchsten Standards enthalten, müssen verdünnt werden. Dieser Verdünnungsfaktor muss bei der Berechnung der Konzentration beachtet werden.

6.3.1 Beispiel für eine Standardkurve

Nachfolgend wird ein typisches Beispiel für eine Standardkurve mit dem DRG ELISA gezeigt. Diese Werte sollten **nicht** zur Berechnung von Patientendaten verwendet werden.

Standard	Optische Dichte (450 nm)
Standard 0 (0 ng/mL)	0,05
Standard 1 (3 ng/mL)	0,23
Standard 2 (10 ng/mL)	0,63
Standard 3 (25 ng/mL)	1,37
Standard 4 (50 ng/mL)	2,35

7 ERWARTETE WERTE

Es wird empfohlen, dass jedes Labor seine eigenen normalen und abnormalen Werte ermittelt.

In einer Studie mit dem DRG TM-CYFRA 21-1 ELISA wurden die Proben von scheinbar gesunden Erwachsenen untersucht. Dabei ergaben sich folgende Werte:

Population	n	Mittelwert (ng/mL)	Median (ng/mL)	5. - 95. Perzentile (ng/mL)	Bereich (min. - max.) (ng/mL)
Männer	121	0,61	0,60	0,10 - 1,33	0,00 - 2,53
Frauen	119	0,58	0,46	0,00 - 1,38	0,00 - 1,98

Mehrere unabhängige Studien empfehlen für CYFRA 21-1 als sogenannten Entscheidungswert (Cut-off) eine Konzentration von 3,3 ng/mL, da alle Patienten ohne Erkrankung und 95 % der Patienten mit benigner Lungenerkrankungen unterhalb diese Wertes liegen (1,2,3).

Die mit diesem Testkit erzielten Ergebnisse sollten niemals als alleinige Grundlage für therapeutische Konsequenzen dienen. Die Ergebnisse müssen zusammen mit anderen klinischen Befunden und diagnostischen Tests des Patienten interpretiert werden.

8 QUALITÄTSKONTROLLE

Es wird empfohlen, die Kontrollproben gemäß den nationalen gesetzlichen Bestimmungen einzusetzen. Durch die Verwendung von Kontrollproben wird eine Tag-zu-Tag Überprüfung der Ergebnisse erzielt. Es sollten Kontrollen sowohl mit normalem als auch pathologischem Level eingesetzt werden.

Die Kontrollen mit den entsprechenden Ergebnissen des QC-Labors sind im QC-Zertifikat, das dem Kit beiliegt, aufgeführt. Die im QC-Blatt angegebenen Werte und Bereiche beziehen sich stets auf die aktuelle Kitcharge und sollten zum direkten Vergleich der Ergebnisse verwendet werden.

Es wird ebenfalls empfohlen, an nationalen oder internationalen Qualitätssicherungs-Programmen teilzunehmen, um die Genauigkeit der Ergebnisse zu sichern.

Es sollten geeignete statistische Methoden zur Analyse von Kontroll-Werten und Trends angewendet werden. Wenn die Ergebnisse des Assays nicht mit den angegebenen Akzeptanzbereichen des Kontrollmaterials übereinstimmen, sollten die Patientenergebnisse als ungültig eingestuft werden.

In diesem Fall überprüfen Sie bitte die folgenden Bereiche: Pipetten und Zeitnehmer, Photometer, Verfallsdatum der Reagenzien, Lagerungs- und Inkubationsbedingungen, Absaug- und Waschmethode.

Sollten Sie nach Überprüfung der vorgenannten Bereiche keinen Fehler erkannt haben, setzen Sie sich bitte mit Ihrem Lieferanten oder direkt mit der Firma DRG in Verbindung.

9 ASSAY-CHARAKTERISTIKA

9.1 Messbereich

Der Messbereich des Testes liegt zwischen 0,079 ng/mL - 50 ng/mL.

9.2 Spezifität der Antikörper (Kreuzreaktivität)

Die Daten entnehmen Sie bitte der ausführlichen englischen Arbeitsanleitung.

9.3 Sensitivität

Der „Limit of Blank“ (LoB) ist 0,079 ng/mL.
Die Nachweisgrenze (LoD) ist 0,185 ng/mL.
Die Quantifizierungsgrenze (LoQ) ist 0,343 ng/mL.

Die Daten zu:

9.4 Reproduzierbarkeit (Präzision)

9.5 Wiederfindung

9.6 Linearität

entnehmen Sie bitte der ausführlichen englischen Version der Gebrauchsanweisung.

10 GRENZEN DES TESTS

Zuverlässige und reproduzierbare Ergebnisse werden erzielt, wenn das Testverfahren mit vollständigem Verständnis der Anweisungen in der Gebrauchsanleitung und unter Befolgung der GLP (Good Laboratory Practice)-Richtlinien durchgeführt wird.

Jede unsachgemäße Behandlung von Proben oder Modifikation dieses Tests können die Ergebnisse beeinflussen.

10.1 Interferenzen

Hämoglobin (bis zu 4 mg/mL), Bilirubin (bis zu 0,5 mg/mL) und Triglyceride (bis zu 7,5 mg/mL) haben keinen Einfluss auf das Testergebnis.

Der Test enthält Reagenzien, um Interferenzen mit HAMA oder heterophilen Antikörpern zu minimieren.

10.2 Beeinflussung durch Medikamente

Bislang sind uns keine Substanzen (Medikamente) bekannt geworden, die einen Einfluss auf die Bestimmung von CYFRA 21-1 in einer Probe haben.

10.3 High-Dose-Hook Effekt

Ein Hook-Effekt tritt in diesem Test bis zu einer Konzentration von 1000 ng/mL CYFRA 21-1 nicht auf

11 RECHTLICHE GRUNDLAGEN

11.1 Zuverlässigkeit der Ergebnisse

Der Test muss exakt gemäß der Testanleitung des Herstellers abgearbeitet werden. Darüber hinaus muss der Benutzer sich strikt an die Regeln der GLP (Good Laboratory Practice) oder andere eventuell anzuwendende Regeln oder nationale gesetzliche Vorgaben halten. Dies betrifft besonders den Gebrauch der Kontrollreagenzien. Es ist sehr wichtig, bei der Testdurchführung stets eine ausreichende Anzahl Kontrollen zur Überprüfung der Genauigkeit und Präzision mitlaufen zu lassen.

Die Testergebnisse sind nur gültig, wenn alle Kontrollen in den vorgegebenen Bereichen liegen, und wenn alle anderen Testparameter die vorgegebenen Spezifikationen für diesen Assay erfüllen. Wenn Sie bezüglich eines Ergebnisses Zweifel oder Bedenken haben, setzen Sie sich bitte mit der Firma DRG in Verbindung.

11.2 Therapeutische Konsequenzen

Therapeutische Konsequenzen sollten keinesfalls nur aufgrund von Laborergebnissen erfolgen, selbst dann nicht, wenn alle Testergebnisse mit den in 11.1 genannten Voraussetzungen übereinstimmen. Jedes Laborergebnis ist nur ein Teil des klinischen Gesamtbildes eines Patienten.

Nur in Fällen, in denen die Laborergebnisse in akzeptabler Übereinstimmung mit dem allgemeinen klinischen Bild des Patienten stehen, sollten therapeutische Konsequenzen eingeleitet werden.

Das Testergebnis allein sollte niemals als alleinige Grundlage für die Einleitung therapeutischer Konsequenzen dienen.

11.3 Haftung

Jegliche Veränderungen des Testkits und/oder Austausch oder Vermischung von Komponenten unterschiedlicher Chargen von einem Testkit zu einem anderen, können die gewünschten Ergebnisse und die Gültigkeit des gesamten Tests negativ beeinflussen. Solche Veränderungen und/oder Austausch haben den Ausschluss jeglicher Ersatzansprüche zur Folge.

Reklamationen, die aufgrund von Falschinterpretation von Laborergebnissen durch den Kunden gemäß Punkt 11.2 erfolgen, sind ebenfalls abzuweisen. Im Falle jeglicher Reklamation ist die Haftung des Herstellers maximal auf den Wert des Testkits beschränkt. Jegliche Schäden, die während des Transports am Kit entstanden sind, unterliegen nicht der Haftung des Herstellers.

1 INTRODUZIONE

Il test immuno-enzimatico **DRG TM-CYFRA 21-1 ELISA** contiene materiale per la determinazione quantitativa di CYFRA 21-1 in siero o plasma (plasma eparina o citrato).

Questo test kit è adatto soltanto per l'uso diagnostico.

2 PRINCIPIO DEL TEST

Il test kit DRG TM-CYFRA 21-1 ELISA è un test immunologico in fase solida con enzimi ancorati su un substrato (ELISA) basato sul **principio sandwich**.

I micropozzetti sono ricoperti con un anticorpo monoclonale diretto contro un unico sito antigenico su una molecola CYFRA 21-1. Un'aliquota di un campione di paziente contenente CYFRA 21-1 endogeno/a viene incubato nel pozzetto ricoperto dell'enzima coniugato, che è un anticorpo anti CYFRA 21-1 monoclonale coniugato alla perossidasi di rafano. Dopo l'incubazione il coniugato non legato è eliminato attraverso lavaggi.

La quantità della perossidasi legata è proporzionale alla concentrazione CYFRA 21-1 nel campione.

Dopo l'aggiunta della soluzione substrato, l'intensità del colore sviluppato è proporzionale alla concentrazione di CYFRA 21-1 nel campione del paziente.

3 PRECAUZIONI

- Questo kit è adatto soltanto per l'uso diagnostico in vitro.
- Si prega di usare la versione valida dell'inserto del pacco a disposizione con il kit.
- Informazioni su sostanze pericolose contenute nel kit sono riportate nel regolamento di sicurezza.
- Tutti i componenti del kit che contengono siero o plasma umano sono controllati e confermati negativi per la presenza di HIV I/II, HbsAg e HCV con metodi conformi alle norme FDA. Ciononostante tutti i componenti dovrebbero essere trattati come potenziali sostanze nocive nella manutenzione e nello smaltimento.
- Il contatto con la *Stop Solution* dovrebbe essere evitato perché contiene 0,5 M H_2SO_4. L'acido solforico può provocare irritazioni cutanee e ustioni.
- Non pipettare con la bocca ed evitare il contatto con componenti del kit con la pelle o con le mucose.
- Nelle aree in cui il test viene utilizzato non fumare, mangiare, bere o fare uso di prodotti cosmetici.
- Nella manutenzione dei campioni o reagenti del kit portare guanti di latex monouso. La contaminazione dei reagenti o dei campioni con microbi può dare risultati falsi.
- L'utilizzo dovrebbe avvenire secondo regole che seguono le rispettive norme di sicurezza nazionali sulle sostanze nocive.
- Non utilizzare i reagenti dopo la scadenza indicata sul kit.
- Ogni indicazione sulla quantità indicata del protocollo del kit deve essere accuratamente seguito. Risultati ottimali possono essere ottenuti soltanto con l'uso di pipette calibrate e spettrofotometro calibrato.
- Componenti del kit con numeri di lotto diversi non devono essere combinati. È consigliabile di non utilizzare pozzetti di piastre diversi, anche se si tratta dello stesso lotto. I kit potrebbero essere stati magazzinati o spediti a condizioni diverse, cosicché le caratteristiche di legame potrebbero divergere leggermente.
- I componenti chimici e reagenti preparati o già utilizzati devono essere trattati e smaltiti secondo le norme di sicurezza nazionali sulle sostanze nocive.
- I regolamenti di sicurezza di questo prodotto possono essere richiesti direttamente dalla ditta DRG Instruments GmbH.

4 COMPONENTI DEL KIT

4.1 Contenuto del kit

1. ***Microtiterwells*** (Micropozetti), 12 x 8 file (separatamente staccabili), 96 pozzetti;
   Pozzetti ricoperti con l'anti-CYFRA 21-1 anticorpo (monoclonale)
2. ***Standard (Standard 0 - 4)***, 5 flaconi, 1 mL ognuno, liofilizzato;
   Concentrazione: 0 - 3 - 10 - 25 - 50 ng/mL
   Vedi "Preparazione dei reagenti".
   Contiene conservante senza mercurio.
3. ***Control Low & High*** (Controllo), 2 flaconi, 1 mL ognuno, liofilizzato;
   I valori dei controlli sono indicati sull'etichetta dei flaconi o sulla descrizione QC.
   vedi „Preparazione dei reagenti"
   Contiene conservante senza mercurio.
4. ***Sample Diluent*** (Diluente dei campioni), 1 flacone, 3 mL, pronto all'uso;
   Contiene conservante senza mercurio.
5. ***Assay Buffer*** *(*Tampone del test), 1 flacone, 7 mL, pronto all'uso;
   Contiene conservante senza mercurio
6. ***Enzyme Conjugate*** (Tracciante enzimatico), 1 flacone, 1,2 mL, pronto all'uso;
   Anti-CYFRA 21-1 anticorpo coniugato alla perossidasi di rafano;
   Contiene conservante senza mercurio.
7. ***Substrate Solution*** (Soluzione di substrato), 1 flacone, 14 mL, pronto all'uso;
   TMB (benzidine tetrametilico).
8. ***Stop Solution*** (Soluzione d'arresto), 1 flacone, 14 mL, pronto all'uso;
   contiene 0,5 M H_2SO_4.
   Evitare il contatto con la soluzione d'arresto. Può causare irritazioni cutanee e ustioni.
9. ***Wash Solution*** (Soluzione di lavaggio), 1 flacone, 30 mL (concentrata 40X);
   vedi „preparazione dei reagenti".

Nota: Ulteriore *Sample Diluent* per la diluizione dei campioni può essere richiesto alla ditta.

4.2 Materiali richiesti ma non contenuti nel kit

- Uno spettrofotometro calibrato per micropozzetti (450 nm ± 10 nm) (p.es. il DRG Instruments Microtiterplate Reader)
- Micropipette calibrate di precisione a volume variabile
- Carta assorbente
- Acqua distillata

4.3 Magazzinaggio e stabilità del kit

A 2 °C a 8 °C i reagenti non aperti rimangono reattivi fino alla data di scadenza indicata. Non usare reagenti oltre questa data.

Tutti i reagenti aperti devono essere magazzinati a 2 °C a 8 °C. I micropozzetti devono essere magazzinati a 2 °C a 8 °C. Una volta aperti i pacchi, questi devono essere richiusi accuratamente.

Test kits aperti rimangono attivi per 8 settimane se magazzinati alle condizioni sopra descritte.

4.4 Preparazione dei reagenti

Prima dell'uso portare tutti i reagenti e il numero necessario di pozzetti a temperatura ambiente.

Standards

Ricostituire il contenuto liofilizzato di ogni flacone con 1 mL acqua distillata e lasciare per almeno 10 minuti a temperatura ambiente. Mescolare alcune volte prima dell'uso.

Nota: *Gli standard ricostituiti sono stabili per 8 settimane a 2 °C a 8 °C.*
Per una conservazione più lunga i standardi ricostituiti devono essere aliquotati e conservati a -20 °C.

Control

Ricostituire il contenuto liofilizzato di ogni flacone con 1 mL acqua distillata e lasciare per almeno 10 minuti a temperatura ambiente. Mescolare alcune volte prima dell'uso.

Nota: *Il controlli ricostituiti sono stabili per 8 settimane a 2 °C a 8 °C.*
Per una conservazione più lunga i controlli ricostituiti devono essere aliquotati e conservati a -20 °C.

Wash Solution

Diluire 30 mL *Wash Solution* concentrata con 1170 mL di acqua deionizzata fino ad un volume finale di 1200 mL.
La soluzione di lavaggio diluita è stabile per 2 settimane a temperatura ambiente.

4.5 Smaltimento del kit

Lo smaltimento del kit deve avvenire secondo le regole a norma di legge. Informazioni particolareggiate per questo prodotto si trovano nel regolamento di sicurezza, capitolo 13.

4.6 Test kits danneggiati

Nel caso di gravi danneggiamenti del kit o dei suoi componenti deve avvenire una dichiarazione scritta alla ditta DRG, al più tardi una settimana dopo il ricevimento del kit. Componenti danneggiati non dovrebbero essere utilizzati per il test. Questi componenti devono essere magazzinati fino ala soluzione del problema. Dopo di che essi devono essere smaltiti secondo le norme ufficiali.

5 CAMPIONI

Siero o plasma (plasma eparina o citrato) può essere usato per questo test.
Il plasma EDTA produce valori aumentati.

Non usare campioni emolitici, itterici o lipemici.
Attenzione: Se i campioni contengono sodio azide non devono essere utilizzati per questo test.

5.1 Collezione dei campioni

Siero:
Collezionare sangue tramite puntura venale (p.es. Sarstedt Monovette per siero), far coagulare e separare il siero centrifugando a temperatura ambiente.
Non centrifugare prima che la coagulazione sia completata. Campioni di pazienti con una terapia anticoagulante possono richiedere più tempo per la coagulazione.

Plasma:
Il sangue dovrebbe essere collezionato in tubetti da centrifuga contenenti un anticoagulante (p. es. Sarstedt Monovette con un'adeguata preparazione per il plasma) e centrifugando immediatamente dopo la puntura.

5.2 Magazzinaggio dei campioni

I campioni dovrebbero essere magazzinati ben chiusi fino a 5 giorni a 2 °C a 8 °C.
Campioni magazzinati per un periodo più lungo (fino a 18 mesi) dovrebbero essere congelati solo una volta a -20 °C prima dell'analisi. Congelare soltanto una volta. Invertire campioni scongelati alcune volte prima dell'uso.

5.3 Diluizione dei campioni

Se in un campione di siero viene trovata una concentrazione oltre lo standard più alto, questo campione può essere diluito con *Sample Diluent* e nuovamente determinato.
Della diluizione deve essere però tenuto conto.

Esempio:
a) diluizione 1:10: 10 µL campione + 90 µL *Sample Diluent* (agitare bene)
b) diluizione 1:100: 10 µL della diluizione a) + 90 µL *Sample Diluent* (agitare bene)

6 ATTUAZIONE DEL TEST

6.1 Indicazioni generali

- Tutti i reagenti e i campioni devono essere portati a temperatura ambiente e ben mescolati prima dell'uso. Evitare la formazione di schiume.
- Una volta iniziato il procedimento del test, questo deve essere portato alla fine senza interruzione.
- Per ogni componente, standard, controllo o campione è necessario utilizzare una nuova punta monouso per evitare reazioni incrociate.
- La densità ottica dipende dal tempo d'incubazione e dalla temperatura. Perciò si rende necessario di preparare tutti i reagenti, di aprire i tappi dei flaconi e di appostare tutti i pozzetti nelle appropriate posizioni. Soltanto una tale preparazione garantisce gli stessi tempi per ogni processo di pipettamento.
- Come regola generale vale che la reazione enzimatica si svolge linearmente proporzionale con il tempo e con la temperatura.

6.2 Eseguimento del test

Ogni analisi deve includere una curva standard.

1. Fissare i pozzetti necessari sul supporto.
2. Pipettare **50 µL** ***Assay Buffer*** in ogni pozzetto.
3. Pipettare **10 µL** ***Enzyme Conjugate*** in ogni pozzetto.
4. Pipettare **50 µL** di ogni ***Standard***, ***Control*** e **campione** nei pozzetti, cambiando ogni volta la punta monouso. Agitare bene per 10 secondi. È molto importante raggiungere un completo mescolamento.
5. Incubare per **60 minuti** a temperatura ambiente.
6. Rovesciare la piastra per vuotare i pozzetti.
   Lavare i pozzetti **3 volte** con **350 µL** *Wash Solution* diluita in ogni pozzetto. Rimuovere le gocce d'acqua rimanenti rivoltando la piastra su carta assorbente.
   Importante:
   La sensibilità e la precisazione di questo kit sono fortemente influenzate dal corretto eseguimento del lavaggio!
7. Aggiungere **100 µL** della ***Substrate Solution*** ad ogni pozzetto.
8. Incubare per **15 minuti** a temperatura ambiente.
9. Fermare la reazione enzimatica aggiungendo **100 µL** della ***Stop Solution*** ad ogni pozzetto.
10. Determinare la densità ottica a **450 ± 10 nm** con un fotometro per microtiter-piastre **entro 10 minuti** dopo l'aggiunta della *Stop Solution*.

6.3 Rilevamento dei risultati

1. Determinare i valori medi della densità ottica per ogni set di standard, controlli e campioni.
2. Costruire una curva standard: riportare i valori medi della densità ottica (OD) di ogni standard contro la rispettiva concentrazione dove i valori delle OD si devono trovare sull'asse verticale (Y) e le concentrazioni sull'asse orizzontale (X).
3. Utilizzando il valore medio delle OD per ogni campione si determina la rispettiva concentrazione dalla curva standard.
4. Metodo automatico: I valori riportati in questo istruzioni per l'uso sono stati determinati tramite l'equazione a 4 parametri. (I methodi preferiti sono 4 Parameter Rodbard oppure 4 Parameter Marquardt.) Altri funzioni usati per l'elaborazioni dei dati possono dare risultati leggermente differenti.
5. La concentrazione dei campioni può essere determinata direttamente dalla curva standard. Campioni con una concentrazione più elevata dello standard più concentrato devono essere diluiti. Di questo fattore di diluizione deve essere tenuto conto per il calcolo della concentrazione.

6.3.1 Esempio di una curva standard tipica

I seguenti dati sono a scopo dimostrativo soltanto e **non possono** sostituire i dati generati dall'eseguimento del test.

Standard	Densità ottiche (450 nm)
Standard 0 (0 ng/mL)	0,05
Standard 1 (3 ng/mL)	0,23
Standard 2 (10 ng/mL)	0,63
Standard 3 (25 ng/mL)	1,37
Standard 4 (50 ng/mL)	2,35

7 VALORI NORMALI

È consigliabile che ogni laboratorio determini i propri valori normali e anormali.

In uno studio condotto con adulti apparentemente sani, usando il DRG TM-CYFRA 21-1 ELISA, i seguenti valori sono stati trovati:

Popolazione	n	Media (ng/mL)	Mediano (ng/mL)	5. - 95. percentile (ng/mL)	Intervallo (min. - max.) (ng/mL)
Uomini	121	0,61	0,60	0,10 - 1,33	0,00 - 2,53
Donne	119	0,58	0,46	0,00 - 1,38	0,00 - 1,98

Diversi studi raccomandano una concentrazione di cut-off di 3.3 ng/mL per CYFRA 21-1, dato che in pazienti non malati e in 95% dei pazienti con una malattia polmonare benigna è stato trovato un valore inferiore a questo valore (1,2,3).

Come per tutti i test diagnostici, una diagnosi clinica definitiva **non** dovrebbe basarsi sui risultati di un singolo dosaggio.
Una diagnosi clinica dovrebbe essere formulata dal medico in seguito ad un'attenta valutazione di tutti gli aspetti clinici assieme ai dati di laboratorio.

8 CONTROLLO QUALITÀ

È consigliabile utilizzare i campioni controllo secondo le norme di legge. Attraverso l'utilizzo dei campioni controllo si può raggiungere una verifica dei risultati giorno per giorno. Dovrebbero essere adoperati campioni controllo sia con un livello normale sia con uno patologico.
Le referenze con i rispettivi risultati del laboratorio QC sono elencati nel QC certificato, che è allegato al kit. I valori riportati nel QC certificato si riferiscono al lotto del kit attuale e dovrebbero essere utilizzati per un raffronto dei risultati.
È altresì consigliabile di partecipare a programmi di sicurezza sulla qualità nazionali o internazionali, per assicurarsi dell'esattezza dei risultati.
Appropriati metodi statistici per l'analisi dei valori controllo e delle rappresentazioni grafici dovrebbero essere adoperati. Nel caso che i risultati del test non combaciano con il campo di accettazione indicato dal materiale di controllo, i risultati dei pazienti devono essere considerati invalidi. In questo caso si prega di controllare i seguenti fattori d'errore: pipette, cronometri, fotometro, data di scadenza dei reagenti, condizione di magazzinaggio e d'incubazione, metodi di aspirazione e di lavaggio.
Se dopo il controllo dei suddetti fattori non è rilevabile alcun errore, si prega di contattare il fornitore o direttamente la ditta DRG.

9 CARATTERISTICHE DEL TEST

9.1 Assay Dynamic Range

Le concentrazioni determinabili con questo test stanno tra 0,079 ng/mL - 50 ng/mL.

9.2 Specificità degli anticorpi (reazioni ad incrocio)

Gli anticorpi utilizzati per il DRG TM-CYFRA 21-1 ELISA sono specifici per Keratin 19.

9.3 Sensitività

Il limite del bianco (LoB) è 0,079 ng/mL.
Il limite di rilevabilità (LoD) è 0,185 ng/mL.
Il limite di quantificazione (LoQ) è 0,343 ng/mL.

Dati dettagliati su

9.4 Precisione
9.5 Recupero
9.6 Linearità

si prega di consultare le dettagliate istruzioni per l'uso in inglese.

10 LIMITAZIONE DEL TEST

Risultati affidabili e riproducibili saranno ottenuti quando il procedimento del test è seguito con una comprensione completa delle istruzioni all'uso e seguendo una buona pratica di laboratorio (GLP).

Ogni manutenzione impropria dei campioni o modificazione al saggio può influenzare i risultati.

10.1 Sostanze interferenti

Emoglobina (fino a 4 mg/mL), bilirubina (fino a 0,5 mg/mL) e trigliceridi (fino a 7,5 mg/mL) non influenzano i risultati di questo test.

Il test contiene reagenti in grado di minimizzare l'interferenza di HAMA e di anticorpi eterofilici.

10.2 Droghe interferenti

Fino ad oggi nessuna sostanza (farmaco) è conosciuta a noi che abbia influenzato la determinazione di CYFRA 21-1 nel campione.

10.3 Effetto Hook (Gancio) ad alto dosaggio

Nessun effetto Hook (gancio) è stato osservato in questo prodotto fino a 1000 ng/mL di CYFRA 21-1.

11 ASPETTI LEGALI

11.1 Affidabilità dei risultati

Il test deve essere eseguito esattamente secondo il protocollo dato dal produttore. Inoltre l'utente deve seguire le regole del GLP (Good Laboratory Practice) o eventualmente altre regole comportamentali o disposizioni legali. Questo vale soprattutto per l'uso delle referenze. È molto importante utilizzare un numero appropriato di referenze in parallelo ai campioni test per poter controllare l'esattezza e la precisione del test.

I risultati del test sono validi soltanto se tutte le referenze cadono nei margini prestabiliti e se tutti gli altri parametri del test soddisfano la specificazione per questo test. Se esistono dubbi o domande su questi risultati, si prega di contattare la ditta DRG.

11.2 Conseguenze terapeutiche

Soltanto sulla base dei risultati dei laboratori non dovrebbero essere intraprese delle conseguenze terapeutiche di alcun tipo, anche se i risultati del test sono d'accordo con gli aspetti articolati nel punto 11.1. Ogni risultato di laboratorio è soltanto una parte di un quadro clinico completo di un paziente.

Soltanto in casi in cui i risultati di un test del laboratorio si accordano con il quadro clinico dell'ammalato, si possono intraprendere delle conseguenze terapeutiche.

Il risultato del test da solo non è base sufficiente per lo stabilimento di una terapia.

11.3 Responsabilità legali

Ogni cambiamento del protocollo del test e/o lo scambio o il mescolamento di componenti provenienti da cariche diverse possono influenzare negativamente i risultati e compromettere la validità del test. Questi cambiamenti e/o scambi annullano ogni diritto al risarcimento.

Si respingano inoltre tutti i richiami risultanti da interpretazioni sbagliate da parte dell'utente secondo il paragrafo 11.2. Nel caso di reclamazione, la garanzia del produttore è limitato al valore massimo del test kit. Ogni danno provocato durante il trasporto del kit non sottostà alla responsabilità del produttore.

1 INTRODUCCIÓN

El **Kit de inmunoensayo enzimático DRG** TM-CYFRA 21-1 ELISA proporciona los materiales necesarios para la determinación cuantitativa del CYFRA 21-1 en suero o plasma (plasma con heparina o citrato).

Este ensayo está diseñado solo para diagnóstico *in vitro*.

2 FUNDAMENTO DEL ENSAYO

El Kit DRG TM-CYFRA 21-1 ELISA es un ensayo en fase sólida de inmunoadsorción unido a enzimas (ELISA), basado en el **principio del sándwich**.

Los pocillos de las placas están recubiertos con un anticuerpo monoclonal/policlonal dirigido contra un único foci antigénico en una molécula de CYFRA 21-1.

Se incuba una alícuota de una muestra perteneciente a un paciente que contiene CYFRA 21-1 endógena en los pocillos recubiertos con el enzima conjugado, que es un anticuerpo anti-CYFRA 21-1 conjugado con la peroxidasa endógena. Después de la incubación se lava el conjugado que no se ha unido.

La cantidad de peroxidasa unida es proporcional a la concentración de CYFRA 21-1 en la muestra.

Cuando se añade la solución del sustrato de la peroxidasa, la intensidad del color desarrollado es proporcional a la concentración de CYFRA 21-1 en la muestra del paciente.

3 PRECAUCIONES

- Este kit es solamente para diagnóstico *in vitro*.
- Por favor, se usa solo la version valida de la metodico técnico incluido aqui en el kit.
- Para obtener información de las sustancias peligrosas incluidas en el kit por favor mirar las hojas de los datos de seguridad del material.
- Todos los reactivos en este kit de ensayo que contienen suero o plasma humano se han ensayado y confirmado ser negativos para HIV I/II, HBsAg y HCV mediante procedimientos aprobados por la FDA. Sin embargo, todos los reactivos deben ser tratados tanto en su uso como dispensación como potencialmente biopeligrosos.
- Evitar contacto con *Stop Solution* que contiene H_2SO_4 0,5 M. Puede provocar irritación y quemaduras en la piel.
- Nunca pipetear con la boca y evitar el contacto de los reactivos y las muestras con la piel y con membranas mucosas.
- No fumar, comer, beber o usar cosméticos en áreas donde las muestras o los reactivos del kit están siendo usados.
- Usar guantes de látex cuando se utilicen las muestras y los reactivos. La contaminación microbiana de los reactivos o las muestras puede dar resultados erróneos.
- El manejo debe realizarse de acuerdo a los procedimientos definidos por las guías o regulación nacionales de seguridad de sustancias biopeligrosas.
- No utilizar los reactivos después de su fecha de caducidad que aparece en las etiquetas del kit.
- Todos los volúmenes indicados han de ser realizados de acuerdo con el protocolo. Los resultados óptimos del ensayo se obtienen solo cuando se utilizan pipetas y lectores de microplacas calibrados.
- No mezclar o usar componentes de kits con distinto número de lote. Se recomienda no intercambiar pocillos de distintas placas incluso si son del mismo lote. Los kits pueden haber sido enviados o almacenados bajo diferentes condiciones y las características de unión de las placas pueden resultar diferentes.
- Los compuestos químicos y los reactivos preparados o utilizados han de tratarse como residuos peligrosos de acuerdo con las guías o regulación nacionales de seguridad de sustancias biopeligrosas.
- Las hojas de los datos de seguridad de este producto están disponibles bajo pedido directamente a DRG Instruments GmbH.

4 COMPONENTES DEL KIT

4.1 Componentes del Kit

1. ***Microtiterwells*** (Placas multipocillo), 12 x 8 tiras separables, 96 pocillos;
   Pocillos recubiertos con anticuerpo anti-CYFRA 21-1 (monoclonal).
2. ***Standard (Standard 0 - 4)***, (Estándar), 5 viales, 1 mL cada, liofilizados;
   Concentraciones: 0 - 3 - 10 - 25 - 50 ng/mL
   Ver “Preparación de los Reactivos”;
   Contiene conservante sin mercurio.
3. ***Control Low & High*** (Control), 2 viales, 1 mL cada, liofilizados;
   Referir los valores y rangos del control a la etiqueta del vial o a la Hoja de datos QC.
   ver “Preparación de los Reactivos”
   Contiene conservante sin mercurio.
4. ***Sample Diluent*** (Solución para dilución de la muestra), 1 vial, 3 mL, listo para usar;
   Contiene conservante sin mercurio.
5. ***Assay Buffer*** (Tampón de ensayo), 1 vial, 7 mL, listo para usar;
   Contiene conservante sin mercurio.
6. ***Enzyme Conjugate*** (Conjugado enzimático), 1 vial, 1,2 mL, listo para usar;
   Anticuerpo anti-CYFRA 21-1 conjugado con la Peroxidasa de rábano;
   Contiene conservante sin mercurio.
7. ***Substrate Solution*** (Solución de sustrato), 1 vial, 14 mL, listo para usar,
   Tetrametilbencidina (TMB).
8. ***Stop Solution*** (Solución de parada), 1 vial, 14 mL, listo para usar,
   contiene 0.5 M H_2SO_4,
   Evitar el contacto con la Solución de parada. Puede causar irritación y quemaduras en al piel.
9. ***Wash Solution*** (Solución de lavado), 1 vial, 30 mL (concentrado 40X),
   ver “Preparación de los Reactivos”.

Nota: Se puede solicitar el *Sample Diluent* para la dilución de la muestra.

4.2 Equipamiento y material requerido pero no provisto

- Lector de microplacas calibrado (450 nm ± 10 nm) (ej. DRG Instruments Microtiter Plate Reader).
- Micropipetas de precisión variable calibradas.
- Papel absorbente.
- Agua destilada.

4.3 Almacenamiento y estabilidad del kit

Cuando se almacena a 2 °C a 8 °C, los reactivos sin abrir mantienen su reactividad hasta la fecha de caducidad. No utilizar los reactivos más allá de esta fecha.

Los reactivos abiertos han de almacenarse a 2 °C a 8 °C. Las placas multipocillo han de almacenarse a 2 °C a 8 °C. Una vez se ha abierto la bolsa hay que tener cuidado y cerrarla de nuevo.

Los kits abiertos conservan su actividad durante 8 semanas si se almacenan como se ha descrito arriba.

4.4 Preparación de los Reactivos

Dejar que todos los reactivos y el número requerido de tiras alcancen la temperatura ambiente antes de usarse.

Standards

Reconstituya el contenido liofilizado de cada tubo con 1 mL de agua destilada y espere al menos 10 minutos a temperatura ambiente. Mezcle varias veces antes de usar.

Nota: *Los estándares reconstituidos son estables durante 8 semanas a 2 °C a 8 °C.*
Para períodos más largos congelar a -20 °C.

Control

Reconstituya el contenido liofilizado de cada tubo con 1 mL de agua destilada y espere al menos 10 minutos a temperatura ambiente. Mezcle varias veces antes de usar.

Nota: *El control reconstituido es estable durante 8 semanas a 2 °C a 8 °C.*
Para períodos más largos congelar a -20 °C.

Wash Solution

Mezclar 30 mL de *Wash Solution* concentrada con 1170 mL de agua desionizada hasta un volumen final de 1200 mL.
La solución del lavado diluida es estable durante 2 semanas a temperatura ambiente.

4.5 Eliminación del Kit

La eliminación del kit debe realizarse de acuerdo con las leyes nacionales. En las hojas de datos de seguridad se proporciona información especial de este producto (ver capítulo 13).

4.6 Kits de ensayo dañados

En caso de que exista cualquier daño severo del kit de ensayo o de sus componentes, ha de informarse por escrito a DRG, no mas tarde de una semana después de recibir el kit. No deben utilizarse componentes dañados para llevar a cabo un ensayo. Han de almacenarse hasta que se encuentre una solución. Después de esto, deben ser eliminados de acuerdo con las leyes oficiales.

5 MUESTRAS

En este ensayo pueden usarse suero o plasma (heparina o citrato).
El plasma EDTA produce valores que se incrementan.

No usar muestras hemolíticas, ictéricas o lipémicas.
Tener en cuenta: No deben usarse muestras que contengan acida sódica.

5.1 Toma de muestras

Suero:
Recoger la sangre por punción en la vena (ej. Sarstedt Monovette para el suero), permitir coagulación, y separar el suero por centrifugación a temperatura ambiente. No centrifugar antes de la coagulación completa. Las muestras de pacientes que reciben terapia anticoagulante requieren más tiempo para coagular.

Plasma:
Toda la sangre ha de recogerse en tubos de centrífuga que contengan anticoagulante (Ej. Sarstedt Monovette con una preparación adecuada para el plasma) y centrifugar inmediatamente tras la recogida.

5.2 Almacenamiento de las muestras

Las muestras deben ser tapadas y pueden ser almacenadas hasta 5 días a 2 °C a 8 °C antes del ensayo.
Las muestras almacenadas por un período de tiempo mas largo (hasta 18 meses) han de congelarse sólo una vez a -20 °C antes del ensayo. Las muestras descongeladas deben invertirse varias veces antes del ensayo.

5.3 Dilución de las muestras

Si en un ensayo inicial, se encuentra una muestra que presenta valores mayores que el estándar mas concentrado, ha de diluirse con *Sample Diluent* y volver a ensayarse como se describe en el Procedimiento de Ensayo.
Para el cálculo de las concentraciones habrá que tener en cuenta el factor de dilución.

Ejemplo:
a) dilución 1:10: 10 µL muestra + 90 µL *Sample Diluent* (mezclar totalmente)
b) dilución 1:100: 10 µL dilución a) 1:10 + 90 µL *Sample Diluent* (mezclar totalmente).

6 PROCEDIMIENTO DE ENSAYO

6.1 Consideraciones generales

- Todos los reactivos y muestras han de estar a temperatura ambiente antes de su uso. Todos los reactivos deben mezclarse sin formar espuma.
- Una vez se ha comenzado el ensayo deben completarse todos los pasos sin interrupción.
- Utilizar puntas de pipeta de plástico nuevas para cada estándar, control o muestra para evitar combinaciones cruzadas.
- La absorbancia es función del tiempo de incubación y la temperatura. Antes de comenzar el ensayo, se recomienda que todos los reactivos estén preparados, tapas removidas, todos los pocillos que se necesiten asegurados en recipiente, etc. Esto asegurará un tiempo similar para cada paso de pipeteo sin que haya interrupciones.
- Como regla general, la reacción enzimática es linealmente proporcional al tiempo y a la temperatura.

6.2 Procedimiento de ensayo

Cada uno debe incluir una curva de estándares.

1. Asegurar el número deseado de pocillos en el recipiente.
2. Dispensar **50 µL** de ***Assay Buffer*** a cada pocillo.
3. Dispensar **10 µL** de ***Enzyme Conjugate*** a cada pocillo.
4. Dispensar **50 µL** de cada ***Standard, Control*** y **muestras** con puntas nuevas en los pocillos adecuados. Mezclar totalmente durante 10 segundos. Es importante mezclar completamente en este paso.
5. Incubar durante **60 minutes** a temperatura ambiente.
6. Sacudir enérgicamente el contenido de los pocillos.
   Lavar los pocillos **3 veces** con **350 µL** *Wash Solution* diluida por pocillo. Realizar un golpe seco de los pocillos contra el papel absorbente para eliminar las gotas residuales.
   Nota importante:
   La sensibilidad y la precisión de este ensayo se ve marcadamente influenciada por la realización correcta del proceso de lavado!
7. Adicionar **100 µL** de ***Substrate Solution*** a cada pocillo.
8. Incubar durante **15 minutes** a temperatura ambiente.
9. Parar la reacción enzimática mediante la adición de **100 µL** de ***Stop Solution*** a cada pocillo.
10. Leer la OD a **450 nm ± 10 nm** con un lector de microplacas **dentro de los 10 minutos** después de la adición de la *Stop Solution*.

6.3 Cálculo de los Resultados

1. Calcular los valores de absorbancia media para cada conjunto de estándares, controles y muestras de pacientes.
2. Construir una curva estándar mediante la representación de la absorbancia media obtenida para cada estándar frente a su concentración con el valor de absorbancia en el eje vertical (Y) y la concentración en el eje horizontal (X).
3. Usando el valor de absorbancia media de cada muestra determinar la concentración correspondiente a partir de la curva estándar.
4. Método automatizado: Los resultados en las instrucciones de uso se han calculado automáticamente usando una curva de regresión 4 Parámetros. (4 Parámetros Rodbard o 4 Parámetros Marquardt son los métodos preferidos.) Otras funciones de regresión darán lugar a resultados sensiblemente diferentes.
5. La concentración de las muestras puede leerse directamente de la curva de estándares. Las muestras con concentraciones superiores al mayor estándar han de diluirse. Para el cálculo de las concentraciones hay que tener en cuenta el factor de dilución.

6.3.1 Ejemplo de una Curva Estándar Típica

Los siguientes datos son solamente para la explicación y **no** pueden ser utilizados en lugar de los datos generados en el momento del ensayo.

Estándar	Unidades Ópticas (450 nm)
Standard 0 (0 ng/mL)	0,05
Standard 1 (3 ng/mL)	0,23
Standard 2 (10 ng/mL)	0,63
Standard 3 (25 ng/mL)	1,37
Standard 4 (50 ng/mL)	2,35

7 VALORES ESPERADOS

Se recomienda encarecidamente que cada laboratorio determine sus valores normales e inusuales.

En un estudio llevado a cabo con adultos aparentemente sanos, usando el DRG TM-CYFRA 21-1 ELISA, se obtuvieron los siguientes valores:

Población	n	Media (ng/mL)	Mediana (ng/mL)	Percentil 5 - 95 (ng/mL)	Rango (min. - max.) (ng/mL)
Hombres	121	0,61	0,60	0,10 - 1,33	0,00 - 2,53
Mujeres	119	0,58	0,46	0,00 - 1,38	0,00 - 1,98

Varios estudios recomendan una concentración del cut-off a 3.3 ng/mL para la CYFRA 21-1, porque todos los pacientes sin enfermedad y un 95 % de pacientes con enfermedades pulmonares benignas se encuentran bajo de esta valor (1,2,3).

Los resultados obtenidos no deberían ser el único motivo para una intervención terapéutica. Los resultados han de correlacionarse con otras observaciones clínicas y tests de diagnóstico.

8 CONTROL DE CALIDAD

Se recomienda usar muestras control de acuerdo con las leyes estatales y federales. El uso de muestras control se recomienda para asegurar la validez diaria de los resultados. Usar controles tanto a niveles normal como patológico.

Los controles y los correspondientes resultados del Laboratorio de control de calidad están fijados en el certificado de control de calidad que acompañan al kit. Los valores y los rangos fijados en la hoja del control de calidad se refieren siempre al kit actual y deben usarse para la comparación directa de los resultados.

Es recomendable también hacer uso de programas de Aseguramiento de la Calidad nacionales o internacionales para asegurar la exactitud de los resultados.

Utilizar métodos estadísticos apropiados para el análisis de los valores y tendencia de los controles. Si los resultados del ensayo no se ajustan a los rangos aceptables establecidos en los controles, los resultados obtenidos de los pacientes han de considerarse inválidos.

En este caso, por favor comprobar las siguientes áreas técnicas: Pipeteo y tiempo empleado, fotómetro, fecha de caducidad de los reactivos, condiciones de almacenamiento e incubación, métodos de aspiración y lavado.

Después de comprobar los asuntos arriba mencionado sin encontrar ningún error, contactar con su distribuidor o con DRG directamente.

9 CARACTERÍSTICAS DEL ENSAYO

9.1 Rango dinámico del ensayo

El rango del ensayo se encuentra entre 0,079 ng/mL - 50 ng/mL.

9.2 Especificidad de los Anticuerpos (Reactividad Cruzada)

Los anticuerpos usados para el ELISA DRG TM-CYFRA 21-1 son específicos para queratina 19.

9.3 Sensibilidad

El límite del blanco (LoB) es 0,079 ng/mL.

El Límite de Detección (LoD) es 0,185 ng/mL.

El Límite de Cuantificación (LoQ) es 0,343 ng/mL.

Para información sobre

9.4 Precisión

9.5 Recuperación

9.6 Linealidad

por favor consulte la versión detallada en inglés de las Instrucciones de Uso.

10 LIMITACIONES DE USO

Únicamente se obtendrán resultados fiables y reproducibles, cuando el procedimiento del ensayo se realice entendiendo las instrucciones de uso correctamente y desarrollando buenas prácticas de laboratorio.

Cualquier manejo impropio de las muestras o modificación del test puede influenciar los resultados.

10.1 Sustancias que pueden interferir

Hemoglobina (hasta 4 mg/mL), Bilirrubina (hasta 0,5 mg/mL) y Triglicéridos (hasta 7,5 mg/mL) no influencian los resultados del ensayo.

El ensayo contiene reactivos para minimizar la interferencia de HAMA y de anticuerpos heterofílicos.

10.2 Interferencias con drogas

Hasta ahora no se han encontrado sustancias (drogas) conocidas por nosotros, que tengan influencia en la medida de CYFRA 21-1 en una muestra.

10.3 Efecto de Alta Concentración (Gancho)

No se ha observado efecto gancho en este ensayo hasta 1000 ng/mL de CYFRA 21-1.

11 ASPECTOS LEGALES

11.1 Fiabilidad de los Resultados

El ensayo debe realizarse exactamente de acuerdo a las instrucciones del fabricante. Mas aún, el usuario debe ajustarse estrictamente a las reglas BPL (Buenas Prácticas de Laboratorio) o a otros estándares y/o leyes nacionales aplicables. Esto es especialmente relevante para el uso de reactivos control. Es importante incluir siempre, dentro del procedimiento de ensayo, un número suficiente de controles para validar la exactitud y la precisión del ensayo.

Los resultados del ensayo son válidos sólo si todos los controles se encuentran dentro de los rangos especificados y si todos los otros parámetros del ensayo se encuentran dentro de las especificaciones dadas para el ensayo. En cado de alguna duda o inquietud, por favor, contactar con DRG.

11.2 Consecuencias Terapéuticas

Las consecuencias terapéuticas nunca deben basarse sólo en los resultados de laboratorio incluso si todos los resultados del ensayo están de acuerdo con los asuntos fijados en el punto 11.1. Cualquier resultado de laboratorio es solamente una parte del cuadro clínico de un paciente.

Solamente en los casos donde los resultados de laboratorio están en acuerdo con todo el cuadro clínico de un paciente, se pueden derivar consecuencias terapéuticas.

Nunca deben derivarse consecuencias terapéuticas a partir de solamente el resultado obtenido en el ensayo

11.3 Responsabilidad

Cualquier modificación del kit y/o cambio o mezcla de cualquier componente procedentes de kits de lotes diferentes puede afectar negativamente a los resultados esperados y en la validez de todo el test. Esas modificaciones y/o cambios invalidad cualquier reclamación de reposición.

Las reclamaciones emitidas debidas a una mala interpretación de los resultados de laboratorio por parte del comprador referidos al punto 11.2 son también inválidas. A pesar de todo, en el caso de cualquier reclamación, la responsabilidad del fabricante no excede el valor del kit. Cualquier daño provocado al kit durante su transporte no está sujeto a la responsabilidad del fabricante.

1 INTRODUCTION

Le kit de dosage immuno-enzymatique **DRG TM-CYFRA 21-1 ELISA** propose les matériaux requis pour la mesure quantitative de CYFRA 21-1 dans le sérum ou du plasma (plasma hépariné ou citraté).

Ce kit est à utiliser uniquement dans le cadre de tests diagnostiques in vitro.

2 PRINCIPE DU TEST

Le kit DRG TM-CYFRA 21-1 ELISA est basé sur une réaction immuno-enzymatique en **sandwich** en phase solide.

Les microplaques sont recouvertes avec un anticorps monoclonal dirigé contre un antigène spécifique de la molécule CYFRA 21-1.

Un aliquot de l'échantillon contenant le (la) CYFRA 21-1 endogène est incubé dans un puits avec l'enzyme conjuguée, c'est-à-dire un anticorps anti-CYFRA 21-1 conjuguée avec la peroxidase de Raifort (horseradish peroxidase, HRP). Apres l'incubation, le conjugué non-lié est éliminé durant le lavage des puits.

La quantité de conjugué-HRP liée est proportionnelle à la concentration de CYFRA 21-1 contenu(e) dans l'échantillon.

Suite à l'addition de solution substrat, l'intensité de la coloration obtenue est proportionnelle à la concentration de CYFRA 21-1 contenu(e) dans l'échantillon.

3 PRECAUTIONS D'UTILISATION

- Ce kit est uniquement destiné aux tests diagnostiques in vitro.
- Utilisez uniquement la version valide d´instructions d'utilisation qui est incluse dans le kit.
- Les informations concernant la toxicité des réactifs contenus dans ce kit sont présentées dans la fiche de sécurité (« Safety Data Sheets »).
- Tous les réactifs de ce kit contenant du sérum ou du plasma humain ont été testés avec des résultats négatifs pour le VIH I/II, le HBsAg et le HCV selon les normes FDA en vigueur. Néanmoins, lors de leur utilisation, tous les réactifs de ce kit doivent être manipulés avec précaution.
- Eviter les contacts avec la *Stop Solution*, celle-ci contient 0,5 M de H_2SO_4. Cela pourrait engendrer irritations ou brûlures de la peau.
- Ne jamais pipeter avec la bouche, et éviter tout contact de la peau ou des muqueuses avec les réactifs ou les échantillons.
- Ne pas fumer, manger, boire ou utiliser des produits cosmétiques dans les zones où les échantillons ou le kit ont été maniés.
- Porter des gants d'examen lors de l'utilisation des échantillons ou des réactifs. Une contamination microbienne des échantillons ou des réactifs pourrait fausser les résultats.
- L'utilisation de ce kit devra être en accord avec les normes ou recommandations nationales de sécurité en vigueur concernant les produits à risque biologique.
- Ne pas utiliser les réactifs au-delà de la date d'expiration inscrite sur l'emballage.
- Tous les volumes indiqués doivent être scrupuleusement respectés, comme indiqué dans le protocole expérimental. Seule l'utilisation de pipettes calibrées ou d'un spectrophotomètre lecteur de micro-plaques calibré garantit l'obtention de résultats optimaux à ce test.
- Ne pas mélanger ou utiliser des réactifs contenus dans des kits de lots différents. Il est conseillé de ne pas échanger les puits de différentes plaques, même si celles-ci proviennent du même lot. Les kits peuvent avoir été transportés ou stockés différemment, et les caractéristiques de liaison de chaque plaque pourraient ainsi être modifiées.
- L'élimination des solutions chimiques et des réactifs contenus dans ce kit, utilisés ou non, doit être en accord avec la réglementation nationale en vigueur concernant l'élimination des déchets à risque biologique.
- La fiche de sécurité concernant ce produit peut être obtenue en contactant directement DRG Instruments GmbH.

4 COMPOSITION DU KIT

4.1 Contenu du kit

1. ***Microtiterwells*** (Plaques de micro-titration), 12 x 8 (à détacher) barrettes, plaques de 96 puits;
   Les puits sont recouverts avec un anticorps anti-CYFRA 21-1 (monoclonal).
2. ***Standard (Standard 0 - 4)***, 5 flacons, 1 mL chacun, lyophilisés;
   Concentrations: 0 - 3 - 10 - 25 - 50 ng/mL
   Voir « Préparation des réactifs ».
   Contient agent de conservation sans mercure.
3. ***Control Low & High*** (Contrôle), 2 flacons, 1 mL chacun, lyophilisés;
   Les valeurs contrôles et limites sont indiquées sur l'étiquette du flacon ou sur la fiche QC.
   Voir « Préparation des réactifs ».
   Contient agent de conservation sans mercure.
4. ***Sample Diluent*** (Solution pour dilution de l'échantillon), 1 flacon, 3 mL, prêt à l'emploi,
   Contient agent de conservation sans mercure.
5. ***Assay Buffer*** (Tampon d'essai)***,***1 flacon, 7 mL, prêt à l'emploi
   Contient agent de conservation sans mercure.
6. ***Enzyme Conjugate*** (Conjugué enzymatique), 1 flacon, 1,2 mL, prêt à l'emploi,
   Anticorps anti-CYFRA 21-1 conjugué à la HRP;
   Contient agent de conservation sans mercure.
7. ***Substrate Solution*** *(Solution substrat)*, 1 flacon, 14 mL, prêt à l'emploi,
   Tétraméthylbenzidine (TMB).
8. ***Stop Solution*** *(Solution d'arrêt)*, 1 flacon, 14 mL, prêt à l'emploi,
   contient 0.5 M de H_2SO_4,
   Eviter les contacts avec la solution stop. Cela pourrait engendrer irritations ou brûlures de la peau.
9. ***Wash Solution*** *(Solution de lavage)*, 1 flacon (concentré 40X),
   voir « Préparation des réactifs ».

Remarque : Un *Sample Diluent* supplémentaire pour la dilution de l'échantillon peut être fourni sur demande.

4.2 Equipement et matériel requis, mais non fournis

- Un spectrophotomètre lecteur de micro-plaques calibré (450 nm ± 10 nm) (ex. le lecteur de microplaques de DRG Instruments GmbH).
- Des micro-pipettes de précision variables et calibrées.
- Du papier absorbant.
- De l'eau distillée.

4.3 Stockage et stabilité du kit

Les réactifs contenus dans des flacons non-ouverts, stockés à 2 °C à 8 °C, seront stables jusqu'à la date d'expiration inscrite sur l'étiquette. Ne pas utiliser les réactifs au delà de cette date.

Les réactifs contenus dans des flacons ouverts doivent être stockés à 2 °C à 8 °C. Les micro-plaques doivent être stockées à 2 °C à 8 °C. Une fois la capsule d'aluminium ouverte, attention à bien refermer le flacon.

Les kits ouverts conservent leur activité durant 8 semaines s'ils sont stockés comme précédemment mentionné.

4.4 Préparation des réactifs

Amener tous les réactifs et le nombre de barrettes nécessaires au test à température ambiante avant utilisation.

Standards

Reconstituer le contenu lyophilisé de chaque flacon avec 1 mL d'eau distillée et attendre au moins 10 minutes à température ambiante. Mélanger plusieurs fois avant utilisation.

Remarque : *Les standards reconstitués sont stables 8 semaines à 2 °C à 8 °C.*
Pour un stockage prolongé, congeler à -20 °C.

Control

Reconstituer le contenu lyophilisé de chaque flacon avec 1 mL d'eau distillée et attendre au moins 10 minutes à température ambiante. Mélanger plusieurs fois avant utilisation.

Remarque : *Le contrôle reconstitué est stable 8 semaines à 2 °C à 8 °C.*
Pour un stockage prolongé, congeler à -20 °C.

Wash Solution

Diluer 30 mL de *Wash Solution* concentrée avec 1170 mL d'eau désionisée, pour un volume final de 1200 mL.

Remarque : *La solution de lavage diluée est stable 2 semaines à température ambiante.*

4.5 Elimination des déchets relatifs au kit

L'élimination des déchets relatifs au kit doit être réalisée selon les règles nationales en vigueur. Les informations spécifiques au kit sont présentées dans la fiche de sécurité (voir chapitre 13).

4.6 Kits endommagés

Dans le cas de dommages importants survenus au kit ou ses composants, informer la DRG, au plus tard une semaine après réception du kit. Les composants endommagés ne doivent pas être utilisés pour le test. Ils doivent être stockés jusqu'à ce qu'une solution adaptée ait été trouvée. Après cela, ils doivent être éliminés selon les directives officielles en vigueur.

5 ECHANTILLON

Sérum ou plasma (Héparine- ou citrate plasma) peuvent être utilisés pour ce test.
Le plasma EDTA conduit à des valeurs augmentées.

Ne pas utiliser des échantillons hémolysés, ictériques ou lipémiques.
Remarque: Les échantillons contenant de l'azide de sodium ne doivent pas être utilisés pour ce test.

5.1 Prélèvement et préparation des échantillons

Sérum:

Prélever le sang par ponction veineuse (ex. Sarstedt Monovette pour sérum), laisser coaguler, puis séparer le sérum par centrifugation à température ambiante. Ne pas centrifuger avant que la coagulation ne soit terminée. Les patients sous traitement anti-coagulant peuvent demander un temps de coagulation plus important.

Plasma:

Le sang total doit être prélevé dans des tubes de centrifugation contenant un anti-coagulant (Sarstedt Monovette avec une préparation appropriée de plasma) et centrifugé immédiatement après le prélèvement.

5.2 Conservation des échantillons

Les tubes contenant les échantillons doivent être fermés et peuvent être stockés jusqu'à 5 jours à 2 °C à 8 °C avant d'être testés.
Les échantillons stockés pour un temps prolongé (jusqu'à 18 mois) doivent être congelés à -20 °C avant d'être testés. Les échantillons décongelés doivent être retournés plusieurs fois avant le test.

5.3 Dilution de l'échantillon

Si, lors d'un test préliminaire, la concentration de l'échantillon se révèle être supérieure à celle du standard le plus concentré, alors l'échantillon doit être dilué avec le *Sample Diluent* et testé de nouveau, comme décrit dans Réalisation du test.
Pour le calcul des concentrations, ce facteur de dilution doit être pris en considération.

Exemple:

a) dilution 1:10: 10 µL de l'échantillon + 90 µL *Sample Diluent* (bien mélanger).
b) dilution 1:100: 10 µL dilution a) 1:10 + 90 µL *Sample Diluent* (bien mélanger).

6 RÉALISATION DU TEST

6.1 Remarques générales

- Tous les réactifs et échantillons doivent être amenés à température ambiante avant utilisation. Tous les réactifs doivent être mélangés, sans formation de mousse.
- Une fois la procédure engagée, toutes les étapes doivent être réalisées sans interruption.
- Utiliser un nouveau cône de pipette pour chaque standard, contrôle ou échantillon, ceci afin d'éviter toute contamination.
- L'absorbance est fonction du temps d'incubation et de la température. Avant de commencer le test, il est recommandé de préparer tous les réactifs, bouchons ouverts, de préparer les puits des microplaques, etc. Cela garantira un intervalle de temps équivalent entre chaque étape, sans interruption.
- En règle générale, la réaction enzymatique est linéairement proportionnelle au temps et à la température.

6.2 Réalisation du dosage

Chaque test doit inclure une courbe étalon.

1. Disposer le nombre de puits de micro-titration désiré dans le support.
2. Déposer **50 µL** ***Assay Buffer*** dans chaque puits.
3. Déposer **10 µL d'*Enzyme Conjugate*** dans chaque puits.
4. Déposer **50 µL** de chaque ***Standard, Control*** et les **échantillons**, avec de nouveaux cônes de pipette, dans les puits appropriés.
   Bien mélanger pendant 10 secondes. Il est important d'obtenir un mélange parfait lors de cette étape.
5. Incuber pendant **60 minutes** à température ambiante.
6. Décanter le contenu des puits et rincer les puits **3 fois** avec **350 µL** de la *Wash Solution* diluée par puits). Tapoter les puits sur du papier absorbant afin d'éliminer les gouttelettes résiduelles.
   Remarque importante:
   La sensibilité et la précision de ce test sont fortement dépendantes de la bonne réalisation des étapes de lavage !
7. Ajouter **100 µL** de ***Substrate Solution*** à chaque puits.
8. Incuber pendant **15 minutes** à température ambiante.
9. Stopper la réaction enzymatique en ajoutant **100 µL** de ***Stop Solution*** à chaque puits.
10. Lire la densité optique à **450 nm ± 10 nm** à l'aide d'un spectrophotomètre lecteur de micro-plaques **dans les 10 minutes** après avoir ajouté la *Stop Solution*.

6.3 Calcul des résultats

1. Calculer les valeurs moyennes des densités optiques pour chaque série de standards, contrôles et échantillons.
2. Etablir la courbe étalon en reportant la densité optique moyenne de chaque valeur standard en fonction de sa concentration, en posant la densité optique en axe des ordonnées et la concentration en axe des abscisses.
3. L'utilisation de la densité optique moyenne pour chaque échantillon détermine la concentration correspondante à partir de la courbe étalon.
4. Méthode automatique. Les résultats dans les instructions d'utilisation ont été calculés de façon automatique en utilisant une courbe de régression 4 Paramètres. (4 paramètres Rodbard ou 4 paramètres Marquardt sont les méthodes favorites.) D'autres fonctions logistiques peuvent donner des résultats légèrement différents.
5. La concentration des échantillons peut être lue directement à partir de cette courbe étalon. Les échantillons avec une concentration supérieure à celle du plus haut standard doivent être dilués de nouveau. Pour le calcul des concentrations, ce facteur de dilution doit être pris en considération.

6.3.1 Exemple d'une courbe standard typique

Les résultats suivants sont ici présentés à titre d'exemple et **ne peuvent** être utilisés au moment de l'essai.

Standard	Unités optiques (450 nm)
Standard 0 (0 ng/mL)	0,05
Standard 1 (3 ng/mL)	0,23
Standard 2 (10 ng/mL)	0,63
Standard 3 (25 ng/mL)	1,37
Standard 4 (50 ng/mL)	2,35

7 VALEURS ATTENDUES

Il est fortement recommandé à chaque laboratoire de déterminer ses propres valeurs normales et pathologiques.

Dans une étude menée sur des adultes apparemment sains, à l'aide du test TM-CYFRA 21-1 ELISA de DRG, les valeurs suivantes ont été observées :

Population	n	Valeur moyenne (ng/mL)	Médiane (ng/mL)	5. - 95. Percentile (ng/mL)	Portée (min. - max.) (ng/mL)
Hommes	121	0,61	0,60	0,10 - 1,33	0,00 - 2,53
Femmes	119	0,58	0,46	0,00 - 1,38	0,00 - 1,98

Pour CYFRA 21-1 plusieurs études indépendantes recommandées comme un « cut-off » une concentration de 3,3 ng/mL, car tous les patients sans maladie et 95% des patients avec une maladie pulmonaire bénignes sont inférieures à cette valeur (1,2,3).

Les résultats ne doivent pas être utilisés seuls pour déterminer les décisions thérapeutiques. Ils doivent être corrélés avec d'autres observations cliniques et tests diagnostiques.

8 CONTROLE DE QUALITE

Il est recommandé d'utiliser les échantillons contrôles selon les réglementations nationales en vigueur. L'utilisation des échantillons contrôles est recommandé afin de s'assurer jour après jour de la validité des résultats. Utiliser les contrôles de valeurs normales et pathologiques.

Les contrôles et les résultats correspondants issus du laboratoire QC sont mentionnés dans le certificat QC fourni avec le kit. Les valeurs et les limites mentionnées sur la fiche QC font toujours référence au lot de kit courant et doivent être utilisées pour une comparaison directe avec les résultats.

Il est également recommandé d'utiliser les programmes d'évaluation de qualité nationaux ou internationaux, afin de s'assurer de l'exactitude des résultats.

Utiliser les méthodes d'analyses statistiques appropriées pour l'analyse des valeurs contrôles et des tendances. Si les résultats ne correspondent pas aux limites établies des contrôles, les résultats concernant ces patients doivent être considérées comme non valides.

Dans ce cas, tester les zones techniques suivantes : mécanisme de pipettage et temps; spectrophotomètre, dates d'expiration des réactifs, conditions de stockage et d'incubation, méthodes d'aspiration et de lavage.

Après avoir tester les points mentionnés, si aucune erreur n'est détectée, contacter votre distributeur ou directement la DRG.

9 CARACTERISTIQUES DU TEST

9.1 Zone de mesure

Les limites du dosage sont comprises entre 0,079 ng/mL - 50 ng/mL.

9.2 Spécificité des anticorps (Réaction croisée)

Les anticorps utilisés pour l'ELISA DRG TM-CYFRA 21-1 sont spécifiques de la kératine 19.

9.3 Sensibilité

La limite du blanc (LoB) est de 0,079 ng/mL.

La limite de détection (LoD) est de 0,185 ng/mL.

La limite de quantification (LoQ) est de 0,343 ng/mL.

Pour

9.4 Précision

9.5 Récupération

9.6 Linéarité

consulter la version anglaise détaillée du mode d'emploi.

10 LIMITES D'UTILISATION

Les résultats seront fiables et reproductibles si la procédure de dosage est effectuée dans le respect le plus strict des instructions et des bonnes pratiques de laboratoire.

Toute manipulation incorrecte des échantillons ou toute modification de ce test peut affecter les résultats.

10.1 Substances parasites

L'hémoglobine (jusqu'à 4 mg/mL), la bilirubine (jusqu'à 0,5 mg/mL) et les triglycérides (jusqu'à 7,5 mg/mL) n'ont aucune influence sur les résultats du dosage.

Le dosage contient des réactifs afin de réduire les interférences des HAMA et des anticorps hétérophiles.

10.2 Drogues parasites

Jusqu'à présent, nous ne connaissons aucune substance (drogues) capable d'influencer la mesure de CYFRA 21-1 dans un échantillon.

10.3 Effet de surdosage

Aucun effet de surdosage n'a été observé pour ce test jusqu'à une concentration de 1000 ng/mL de CYFRA 21-1.

11 ASPECTS LEGAUX

11.1 Fiabilité des résultats

Ce test doit être exactement utilisé selon les instructions d'utilisation du fabricant. De plus, les utilisateurs doivent strictement respecter les règles de la bonne pratique de laboratoire, ou autres lois nationales. Cela est spécialement le cas pour l'utilisation des réactifs contrôles. Pour chaque test, il est important d'inclure un nombre suffisant de contrôles, afin de pouvoir valider l'exactitude et la précision du test.

Les résultats du test sont valides si et seulement si tous les contrôles sont compris dans les gammes de mesure mentionnées et si tous les autres paramètres du test sont également compris dans les instructions de ce test. En cas de doute ou d'inquiétude, contacter la DRG.

11.2 Conséquences thérapeutiques

Les suites thérapeutiques ne devront jamais être basées sur les résultats de laboratoire seuls, même si les tous les résultats du test sont en accord avec les points mentionnés dans le paragraphe 11.1. Tout résultat n'est qu'une partie du tableau clinique complet d'un patient.

Les suites thérapeutiques peuvent découler des résultats de laboratoire si et seulement si ceux-ci sont en accord avec l'ensemble du tableau clinique du patient.

Le résultat du test en lui-même ne doit en aucun cas être le seul déterminant des suites thérapeutiques à suivre.

11.3 Responsabilité

Toute modification du kit et / ou échange ou mélange d'un des composants de différents lots, d'un kit à un autre, pourrait affecter de façon négative les résultats attendus et la validité du test dans son ensemble. De telles modifications ou échanges invalident toute réclamation pour remplacement.

Toutes les réclamations soumises, relatives au paragraphe 11.2, et dues à une mauvaise interprétation des résultats de laboratoire de la part du client sont également invalides. Néanmoins, en cas de réclamation, la responsabilité du fabricant n'est pas de dépasser les limites de la valeur du kit. Tout dommage causé au kit lors de son transport n'est pas du ressort de la responsabilité du fabricant.

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    J. Urol.; 2005; 174(5); 1771-5.

SYMBOLS USED

Symbol	English	Deutsch	Italiano	Español	Français
CE	European Conformity	CE-Konformitäts-kennzeichnung	Conformità europea	Conformidad europea	Conformité normes européennes
	Consult instructions for use *	Gebrauchsanweisung beachten *	Consultare le istruzioni per l'uso	Consulte las instrucciones de uso	Consulter les instructions d'utilisation
IVD	*In vitro* diagnostic medical device *	*In-vitro*-Diagnostikum *	Diagnostica in vitro	Diagnóstico in vitro	Diagnostic in vitro
REF	Catalogue number *	Artikelnummer *	No. di Cat.	No de catálogo	Référence
LOT	Batch code *	Chargencode *	Lotto no	Número de lote	No. de lot
	Contains sufficient for <n> tests *	Ausreichend für <n> Prüfungen *	Contenuto sufficiente per "n" saggi	Contenido suficiente para <n> ensayos	Contenu suffisant pour "n" tests
	Temperature limit *	Temperaturbegrenzung *	Temperatura di conservazione	Temperatura de conservacion	Température de conservation
	Use-by date *	Verwendbar bis *	Data di scadenza	Fecha de caducidad	Date limite d'utilisation
	Manufacturer *	Hersteller *	Fabbricante	Fabricante	Fabricant
	Caution *	Achtung *			
RUO	For research use only	Nur für Forschungszwecke	Solo a scopo di ricerca	Sólo para uso en investigación	Seulement dans le cadre de recherches
Distributed by	Distributed by	Vertreiber	Distributore	Distribuidor	Distributeur
Content	Content	Inhalt	Contenuto	Contenido	Conditionnement
Volume/No.	Volume / No.	Volumen / Anzahl	Volume / Quantità	Volumen / Número	Volume / Quantité
Microtiterwells	Microtiterwells	Mikrotiterwells	Micropozzetti	Placas multipocillo	Plaques de micro-titration
Antiserum	Antiserum	Antiserum	Antisiero	Antisuero	Antisérum
Enzyme Conjugate	Enzyme Conjugate	Enzymkonjugat	Tracciante enzimatico	Conjugado enzimático	Conjugué enzymatique
Enzyme Complex	Enzyme Complex	Enzymkomplex	Complesso enzimatico	Complejo enzimático	Complexe enzymatique
Substrate Solution	Substrate Solution	Substratlösung	Soluzione di substrato	Solución de sustrato	Solution substrat
Stop Solution	Stop Solution	Stopplösung	Soluzione d´ arresto	Solución de parada	Solution d'arrêt
Zero Standard	Zero Standard	Nullstandard	Standard zero	Estándar cero	Zero Standard
Standard	Standard	Standard	Standard	Estándar	Standard
Control	Control	Kontrolle	Controllo	Control	Contrôle
Assay Buffer	Assay Buffer	Assaypuffer	Tampone del test	Tampón de ensayo	Tampon d'essai
Wash Solution	Wash Solution	Waschlösung	Soluzione di lavaggio	Solución de lavado	Solution de lavage
1N NaOH	1N NaOH	1N NaOH	1N NaOH (idrossido di sodio 1N)	1N NaOH	1N NaOH
1 N HCl	1 N HCl	1 N HCl		1 N HCl	1N HCl
Sample Diluent	Sample Diluent	Probenverdünnungs-medium	Diluente dei campioni	Solución para dilución de la muestra	Solution pour dilution de l'échantillon
Conjugate Diluent	Conjugate Diluent	Konjugatverdünnungs-medium	Diluente del tracciante	Solución para dilución del conjugado	Solution pour dilution du conjugué

HBe Ag&Ab

Enzyme Immunoassay (ELISA) for the determination of Hepatitis B Virus "e" Antigen and Antibody in human plasma and sera.

- for “in vitro” diagnostic use only -

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REF. HBE.CE
96 Tests

HBe Ag&Ab

A. INTENDED USE

Enzyme ImmunoAssay (ELISA) for the determination of Hepatitis B Virus "e" Antigen and Antibody in human plasma and sera.

The kit is intended for the follow-up of acute infection and of chronic patients under therapy.

For “in vitro” diagnostic use only.

B. INTRODUCTION

Hepatitis B “e” Antigen or HBeAg is known to be intimately associated with Hepatitis B Virus or HBV replication and the presence of infectious Dane particles in the blood.

Recently, it has been found that HBeAg is a product of proteolytic degradation of Hepatitis B core Antigen or HBcAg, occurring in hepatocites, whose expression is under the control of the precore region of HBV genome.

If HBeAg is considered a specific marker of infectivity, the presence of anti HBeAg antibodies in blood is recognised to be a clinical sign of recovery from infection to convalescence.

The determination of these two analytes in samples from HBV patients has become important for the classification of the phase of illness and as a prognostic value in the follow up of infected patients.

C. PRINCIPLE OF THE TEST

HBeAg:

HBeAg, if present in the sample, is captured by a specific monoclonal antibody, in the 1st incubation.

In the 2nd incubation, after washing, a tracer, composed of a mix of two specific anti HBeAg monoclonal antibodies, labeled with peroxidase (HRP), is added to the microplate and binds to the captured HBeAg.

The concentration of the bound enzyme on the solid phase is proportional to the amount of HBeAg in the sample and its activity is detected by adding the chromogen/substrate in the 3rd incubation.

The presence of HBeAg in the sample is determined by means of a cut-off value that allows for the semiquantitative detection of the antigen.

HBeAb

Anti HBeAg antibodies, if present in the sample, compete with a recombinant HBeAg preparation for a fixed amount of an anti HBeAg antibody, coated on the microplate wells.

The competitive assay is carried out in two incubations, the first with the sample and recHBeAg, and the second with a tracer, composed of two anti HBeAg monoclonal antibodies, labeled with peroxidase (HRP).

The concentration of the bound enzyme on the solid phase becomes inversely proportional to the amount of anti HBeAg antibodies in the sample and its activity is detected by adding the chromogen/substrate in the third incubation.

The concentration of HBeAg specific antibodies in the sample is determined by means of a cut-off value that allows for the semi quantitative detection of anti HBeAg antibodies.

D. COMPONENTS

The kit contains reagents for total 96 tests.

1. Microplate: MICROPLATE

n° 1 coated microplate

12 strips of 8 breakable wells coated with anti HBeAg specific monoclonal antibody, postcoated with bovine serum proteins and sealed into a bag with desiccant. Allow the microplate to reach room temperature before opening; reseal unused strips in the bag with desiccant and store at 2..8°C.

2. Negative Control: CONTROL -

1x2.0ml/vial. Ready to use control. It contains bovine serum, 0.09% sodium azide and 0.045% ProClin 300 as preservatives. The negative control is colorless.

3. Antigen Positive Control: CONTROL + Ag

1x1.0ml/vial. Ready to use control. It contains 2% bovine serum albumin, non infectious recombinant HBeAg, 100 mM tris buffer pH 7.4+/-0.1, 0.09% sodium azide and 0.045% ProClin 300 as preservatives.

The positive control is green color coded.

4. Antibody Positive Control: **:** CONTROL + Ab

1x1.0ml/vial. Ready to use control. It contains 2% bovine serum albumin, human anti HbeAg positive plasma at about 10 PEI U/ml, 100 mM tris buffer pH 7.4+/-0.1, 0.09% sodium azide and 0.045% ProClin 300 as preservatives. The label is red colored.

The positive control is yellow color coded.

5. Antigen Calibrator: CALAG …ml

n° 1 vial. Lyophilised calibrator for HBeAg. To be dissolved with EIA grade water as reported in the label. It contains fetal bovine serum, non infectious recombinant HBeAg at 1 PEI U/ml +/- 10%, 0.02% gentamicine sulphate and 0.045% ProClin 300 as preservatives.

Important Note: The volume necessary to dissolve the content of the vial may vary from lot to lot. Please use the right volume reported on the label.

6. Antibody Calibrator: CALAB …ml

n° 1 vial. Lyophilized calibrator for anti HBeAg antibody. To be dissolved with EIA grade water as reported in the label. It contains fetal bovine serum, positive plasma at 0.25 PEI U/ml +/-10%, 0.02% gentamicine sulphate and 0.045% ProClin 300 as preservatives. The label is red colored.

Important Note: The volume necessary to dissolve the content of the vial may vary from lot to lot. Please use the right volume reported on the label.

7. Wash buffer concentrate: WASHBUF 20X

1x60ml/bottle. 20x concentrated solution.

Once diluted, the wash solution contains 10 mM phosphate buffer pH 7.0+/-0.2, 0.05% Tween 20 and 0.045% ProClin 300.

8. Enzyme conjugate: CONJ

1x16ml/vial. Ready to use conjugate. It contains Horseradish peroxidase conjugated with a mix of monoclonal antibodies to HBeAg, 10 mM Tris buffer pH 6.8+/-0.1, 2% BSA, 0.045% ProClin 300 and 0.02% gentamicine sulphate as preservatives.

The reagent is red color coded.

9. HBe Antigen: Ag-HBe

1x10ml/vial. Ready to use reagent. It contains recombinant HBeAg, fetal bovine serum, buffered solution pH 8.0+/-0.1, 0.045% ProClin 300 and 0.09% sodium azide as preservatives.

The reagent is blue color coded.

10. Chromogen/Substrate: SUBS TMB

1x16ml/vial. Ready-to-use component. It contains a 50 mM citrate-phosphate buffered solution at pH 3.5-3.8, 4% dimethylsulphoxide, 0.03% tetra-methyl-benzidine or TMB and 0.02% hydrogen peroxide or H_2O_2.

Note: To be stored protected from light as sensitive to strong illumination.

11. Sulphuric Acid: H_2SO_4 O.3 M

1x15ml/vial. It contains 0.3 M H2SO4 solution.

Attention: Irritant (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

12. Plate sealing foils n°2

13. Package insert n°1

E. MATERIALS REQUIRED BUT NOT PROVIDED

1. Calibrated Micropipettes (150ul, 100ul and 50ul) and disposable plastic tips.
2. EIA grade water (double distilled or deionised, charcoal treated to remove oxidizing chemicals used as disinfectants).
3. Timer with 60 minute range or higher.
4. Absorbent paper tissues.
5. Calibrated ELISA microplate thermostatic incubator (dry or wet) set at +37°C.
6. Calibrated ELISA microwell reader with 450nm (reading) and with 620-630nm (blanking) filters.
7. Calibrated ELISA microplate washer.
8. Vortex or similar mixing tools.

F. WARNINGS AND PRECAUTIONS

1. The kit has to be used by skilled and properly trained technical personnel only, under the supervision of a medical doctor responsible of the laboratory.
2. All the personnel involved in performing the assay have to wear protective laboratory clothes, talc-free gloves and glasses. The use of any sharp (needles) or cutting (blades) devices should be avoided. All the personnel involved should be trained in biosafety procedures, as recommended by the Center for Disease Control, Atlanta, U.S. and reported in the National Institute of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
3. All the personnel involved in sample handling should be vaccinated for HBV and HAV, for which vaccines are available, safe and effective.
4. The laboratory environment should be controlled so as to avoid contaminants such as dust or air-born microbial agents, when opening kit vials and microplates and when performing the test. Protect the Chromogen/Substrate (TMB) from strong light and avoid vibration of the bench surface where the test is undertaken.
5. Upon receipt, store the kit at 2-8°C into a temperature controlled refrigerator or cold room.
6. Do not interchange components between different lots of the kits. It is recommended that components between two kits of the same lot should not be interchanged.
7. Check that the reagents are clear and do not contain visible heavy particles or aggregates. If not, advise the laboratory supervisor to initiate the necessary procedures.
8. Avoid cross-contamination between serum/plasma samples by using disposable tips and changing them after each sample. Do not reuse disposable tips.
9. Avoid cross-contamination between kit reagents by using disposable tips and changing them between the use of each one. Do not reuse disposable tips.
10. Do not use the kit after the expiration date stated on external (primary container) and internal (vials) labels.
11. Treat all specimens as potentially infective. All human serum specimens should be handled at Biosafety Level 2, as recommended by the Center for Disease Control, Atlanta, U.S. in compliance with what reported in the Institutes of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
12. The use of disposable plastic-ware is recommended in the preparation of the washing solution or in transferring components into other containers of automated workstations, in order to avoid contamination.
13. Waste produced during the use of the kit has to be discarded in compliance with national directives and laws concerning laboratory waste of chemical and biological substances. In particular, liquid waste generated from the washing procedure, from residuals of controls and from samples has to be treated as potentially infective material and inactivated. Suggested procedures of inactivation are treatment with a 10% final concentration of household bleach for 16-18 hrs or heat inactivation by autoclave at 121°C for 20 min..
14. Accidental spills have to be adsorbed with paper tissues soaked with household bleach and then with water. Tissues should then be discarded in proper containers designated for laboratory/hospital waste.
15. The Stop Solution is an irritant. In case of spills, wash the surface with plenty of water
16. Other waste materials generated from the use of the kit (example: tips used for samples and controls, used microplates) should be handled as potentially infective and disposed according to national directives and laws concerning laboratory wastes.

G. SPECIMEN: PREPARATION AND RECOMMANDATIONS

1. Blood is drawn aseptically by venepuncture and plasma or serum is prepared using standard techniques of preparation of samples for clinical laboratory analysis. No influence has been observed in the preparation of the sample with citrate, EDTA and heparin.
2. Avoid any addition of preservatives; especially sodium azide as this chemical would affect the enzymatic activity of the conjugate, generating false negative results.
3. Samples have to be clearly identified with codes or names in order to avoid misinterpretation of results.
4. Haemolysed and visibly hyperlipemic ("milky") samples have to be discarded as they could generate false results. Samples containing residues of fibrin or heavy particles or microbial filaments and bodies should be discarded as they could give rise to false results.
5. Sera and plasma can be stored at +2°…+8°C in primary collection tubes for up to five days after collection.
Do not freeze primary tubes of collection. For longer storage periods, sera and plasma samples, carefully removed from the primary collection tube, can be stored frozen at –20°C for at least 12 months. Any frozen samples should not be frozen/thawed more than once as this may generate particles that could affect the test result.
6. If particles are present, centrifuge at 2.000 rpm for 20 min or filter using 0.2-0.8u filters to clean up the sample for testing.

H. PREPARATION OF COMPONENTS AND WARNINGS

A study conducted on an opened kit has not pointed out any relevant loss of activity up to 6 re-uses of the device and up to 3 months.

1. Microplate:
Allow the microplate to reach room temperature (about 1 hr) before opening the container. Check that the desiccant has not turned dark green, indicating a defect in manufacturing.
In this case, call Dia.Pro's customer service.
Unused strips have to be placed back into the aluminum pouch, with the desiccant supplied, firmly zipped and stored at +2°-8°C.
When opened the first time, unused strips are stable until the humidity indicator inside the desiccant bag turns from yellow to green.

2. Negative Control:
Ready to use. Mix well on vortex before use.

3. Antigen Positive Control:
Ready to use. Mix well on vortex before use.

4. Antibody Positive Control:
Ready to use. Mix well on vortex before use.

5. Antigen Calibrator:
Add the volume of ELISA grade water, reported on the label, to the lyophilized powder; let fully dissolve and then gently mix on vortex.

Note: The dissolved calibrator is not stable. Store it frozen in aliquots at –20°C.

6. Antibody Calibrator:
Add the volume of ELISA grade water, reported on the label, to the lyophilized powder; let fully dissolve and then gently mix on vortex.
Note: The dissolved calibrator is not stable. Store it frozen in aliquots at –20°C.

7. Wash buffer concentrate:
The whole content of the 20x concentrated solution has to be diluted with bidistilled water up to 1200 ml and mixed gently end-over-end before use.
During preparation avoid foaming as the presence of bubbles could impact on the efficiency of the washing cycles.
Note: Once diluted, the wash solution is stable for 1 week at +2..8° C.

8. Enzyme conjugate:
Ready to use. Mix well on vortex before use.
Avoid contamination of the liquid with oxidizing chemicals, air-driven dust or microbes. If this component has to be transferred, use only plastic, and if possible, sterile disposable containers.

9. HBe Antigen:
Ready to use. Mix well on vortex before use.
Avoid contamination of the liquid with oxidizing chemicals, air-driven dust or microbes. If this component has to be transferred, use only plastic, and if possible, sterile disposable containers.

10. Chromogen/Substrate:
Ready to use. Mix well on vortex before use.
Avoid contamination of the liquid with oxidizing chemicals, air-driven dust or microbes. Do not expose to strong light, oxidizing agents and metallic surfaces.
If this component has to be transferred use only plastic, and if possible, sterile disposable container.

11. Sulphuric Acid:
Ready to use. Mix well on vortex before use.
Attention: Irritant (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

Legenda:

Warning **H statements:**
H315 – Causes skin irritation.
H319 – Causes serious eye irritation.

Precautionary **P statements:**
P280 – Wear protective gloves/protective clothing/eye protection/face protection.

P302 + P352 – IF ON SKIN: Wash with plenty of soap and water.
P332 + P313 – If skin irritation occurs: Get medical advice/attention.
P305 + P351 + P338 – IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P337 + P313 – If eye irritation persists: Get medical advice/attention.
P362 + P363 – Take off contaminated clothing and wash it before reuse.

I. INSTRUMENTS AND TOOLS USED IN COMBINATION WITH THE KIT

1. Micropipettes have to be calibrated to deliver the correct volume required by the assay and must be submitted to regular decontamination (household alcohol, 10% solution of bleach, hospital grade disinfectants) of those parts that could accidentally come in contact with the sample. Decontamination of spills or residues of kit components should also be carried out regularly. They should also be regularly maintained in order to show a precision of 1% and a trueness of $\pm$2%.
2. The ELISA incubator has to be set at +37°C (tolerance of +/- 0.5°C) and regularly checked to ensure the correct temperature is maintained. Both dry incubators and water baths are suitable for the incubations, provided that the instrument is validated for the incubation of ELISA tests.
3. The **ELISA washer** is extremely important to the overall performances of the assay. The washer must be carefully validated in advance, checked for the delivery of the right dispensation volume and regularly submitted to maintenance according to the manufacturer's instructions for use. In particular the washer, at the end of the daily workload, has to be extensively cleaned out of salts with deionized water. Before use, the washer has to be extensively primed with the diluted Washing Solution.
   The instrument weekly has to be submitted to decontamination according to its manual (NaOH 0.1 M decontamination suggested).
   5 washing cycles (aspiration + dispensation of 350ul/well of washing solution + 20 sec soaking = 1 cycle) are sufficient to ensure the assay with the declared performances. If soaking is not possible add one more cycle of washing.
   An incorrect washing cycle or salt-blocked needles are the major cause of false positive reactions.
4. Incubation times have a tolerance of $\pm$5%.
5. The ELISA reader has to be equipped with a reading filter of 450nm and with a second filter of 620-630nm, mandatory for blanking purposes. Blanking is carried out on the well identified in the section "Assay Procedure". The optical system of the reader has to be calibrated regularly to ensure the correct optical density is measured. It should be regularly maintained according to the manufacturer 's instructions.
6. When using an ELISA automated work station, all critical steps (dispensation, incubation, washing, reading, data handling) have to be carefully set, calibrated, controlled and regularly serviced in order to match the values reported in the section "Internal Quality Control''. The assay protocol has to be installed in the operating system of the unit and validated as for the washer and the reader. In addition, the liquid handling part of the station (dispensation and washing) has to be validated and correctly set. Particular attention must be paid to avoid carry over by the needles used for dispensing and for washing. This must be studied and controlled to minimize the possibility of contamination of adjacent wells. The use of ELISA automated work stations is recommended when the number of samples to be tested exceed 20-30 units per run.
7. Dia.Pro's customer service offers support to the user in the setting and checking of instruments used in combination with the kit, in order to assure compliance with the requirements described. Support is also provided for the installation of new instruments to be used with the kit.

L. PRE ASSAY CONTROLS AND OPERATIONS

1. Check the expiration date of the kit printed on the external label (primary container). Do not use if expired.
2. Check that the liquid components are not contaminated by visible particles or aggregates. Check that the Chromogen/Substrate (TMB+H_2O_2) is colourless or pale blue by aspirating a small volume of it with a sterile plastic pipette. Check that no breakage occurred in transportation

and no spillage of liquid is present inside the box (primary container). Check that the aluminium pouch, containing the microplate, is not punctured or damaged.
3. Dilute all the content of the 20x concentrated Wash Solution as described above.
4. Dissolve the Calibrator as described above and gently mix.
5. Allow all the other components to reach room temperature (about 1 hr) and then mix gently on vortex all liquid reagents.
6. Set the ELISA incubator at +37°C and prepare the ELISA washer by priming with the diluted washing solution, according to the manufacturers instructions. Set the right number of washing cycles as reported in the specific section.
7. Check that the ELISA reader is turned on or ensure it will be turned on at least 20 minutes before reading.
8. If using an automated work station, turn on, check settings and be sure to use the right assay protocol.
9. Check that the micropipettes are set to the required volume.
10. Check that all the other equipment is available and ready to use.

In case of problems, do not proceed further with the test and advise the supervisor.

M. ASSAY PROCEDURE

The assay has to be performed according to the procedure given below, taking care to maintain the same incubation time for all the samples being tested.

A) HBe Antigen:

1. Place the required number of strips in the plastic holder and carefully identify the wells for controls, calibrator and samples.
2. Leave the A1 well empty for blanking purposes.
3. Pipette 100 µl of the Negative Control in triplicate, 100 µl of the Antigen Calibrator in duplicate and then 100 µl of the Antigen Positive Control in single.
4. Then dispense 100 µl of samples in the proper wells.
5. Check for the presence of samples in wells by naked eye (there is a marked colour difference between empty and full wells) or by reading at 450/620nm (samples show OD values higher than 0.100).
6. Incubate the microplate for **60 min at +37°C**.

Important note: *Strips have to be sealed with the adhesive sealing foil, only when the test is performed manually. Do not cover strips when using ELISA automatic instruments.*

7. When the first incubation is finished, wash the microwells as previously described (section I.3)
8. Dispense 100 µl Enzyme Conjugate in all wells, except for A1, used for blanking operations.

Important note: *Be careful not to touch the inner surface of the well with the pipette tip and not to immerse the top of it into samples or controls. Contamination might occur.*

9. Check that the reagent has been dispensed properly and then incubate the microplate for **60 min at +37°C**.
10. When the second incubation is finished, wash the microwells as previously described (section I.3)
11. Pipette 100 µl Chromogen/Substrate into all the wells, A1 included.

Important note: *Do not expose to strong direct light as a high background might be generated.*

12. Incubate the microplate protected from light at **room temperature (18-24°C) for 20 minutes**. Wells dispensed with positive control and positive samples will turn from clear to blue.
13. Pipette 100 µl Sulphuric Acid into all the wells using the same pipetting sequence as in step 11. Addition of the stop solution will turn the positive control and positive samples from blue to yellow.
14. Measure the color intensity of the solution in each well, as described in section I.5 using a 450nm filter (reading) and a 620-630nm filter (background subtraction, mandatory), blanking the instrument on A1.

B) HBe Antibody:

1. Place the required number of strips in the plastic holder and carefully identify the wells for controls, calibrator and samples.
2. Leave the A1 well empty for blanking purposes.
3. Pipette 50 µl of the Negative Control in triplicate, 50 µl of the Antibody Calibrator in duplicate and then 50 µl of the Antibody Positive Control in single.
4. Then dispense 50 µl of samples in the proper wells.
5. Check for the presence of samples in wells by naked eye (there is a marked color difference between empty and full wells) or by reading at 450/620nm (samples show OD values higher than 0.100).
6. Dispense then 50 µl of HBe Antigen in all the wells, except for A1.
7. Incubate the microplate for **60 min at +37°C**.

Important note: *Strips have to be sealed with the adhesive sealing foil, only when the test is performed manually. Do not cover strips when using ELISA automatic instruments.*

8. When the first incubation is finished, wash the microwells as previously described (section I.3)
9. Finally proceed as described for the HBeAg assay from point 8 to the last one.

Important notes:

1. *Ensure that no finger prints are present on the bottom of the microwell before reading. Finger prints could generate false positive results on reading.*
2. *Reading should ideally be performed immediately after the addition of the Stop Solution but definitely no longer than 20 minutes afterwards. Some self oxidation of the chromogen can occur leading to a higher background.*
3. *The Calibrator (CAL) does not affect the cut-off calculation and therefore the test results calculation. The Calibrator may be used only when a laboratory internal quality control is required by the management.*

N. ASSAY SCHEME

HBe antigen test

Controls and calibrator Samples	100 ul 100 ul
1st incubation	**60 min**
Temperature	+37°C
Wash step	n° 5 cycles with 20'' of soaking OR n° 6 cycles without soaking
Enzyme Conjugate	100 ul
2nd incubation	**60 min**
Temperature	+37°C
Wash step	n° 5 cycles with 20'' of soaking OR n° 6 cycles without soaking
TMB/H2O2 mix	100 ul
3rd incubation	**20 min**
Temperature	r.t.
Sulphuric Acid	100 ul
Reading OD	450nm/620-630nm

HBe antibody test

Controls and calibrator Samples	50 ul 50 ul
Neutralising antigen	50 ul
1st incubation	**60 min**
Temperature	+37°C
Wash step	n° 5 cycles with 20'' of soaking OR n° 6 cycles without soaking
Enzymatic conjugate	100 ul
2nd incubation	**60 min**
Temperature	+37°C
Wash step	n° 5 cycles with 20'' of soaking OR n° 6 cycles without soaking
TMB/H2O2 mixture	100 ul
3rd incubation	**20 min**
Temperature	r.t.
Sulphuric Acid	100 ul
Reading OD	450nm/620-630nm

An example of dispensation scheme is reported below:

Microplate

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	S2										
B	NC	S3										
C	NC	S4										
D	NC	S5										
E	CAL	S6										
F	CAL	S7										
G	PC	S8										
H	S1	S9										

Legenda: BLK = Blank // NC = Negative Control
PC = Positive Control // CAL = Calibrators // S = Sample

O. INTERNAL QUALITY CONTROL

A validation check is carried out on the controls any time the kit is used in order to verify whether the performances of the assay are as qualified.

Control that the following data are matched:

HBe Antigen

Check	OD450nm
Blank well	< 0.100 OD450nm
Negative Control (NC)	< 0.150 OD450nm after blanking coefficient of variation < 30%
Antigen Calibrator	S/Co > 2.0
Positive Control (PC)	> 1.500 OD450nm

HBe Antibody

Check	OD450nm
Blank well	< 0.100 OD450nm
Negative Control (NC)	> 1.000 OD450nm after blanking coefficient of variation < 10%
Antibody Calibrator	OD450nm < NC/1.5
Positive Control (PC)	OD450nm < NC/10

If the results of the test match the requirements stated above, proceed to the next section.

If they do not, don't proceed any further and perform the following checks:

HBeAg

Problem	Check
Blank well > 0.100 OD450nm	1. that the Chromogen/Substrate solution has not become contaminated during the assay
Negative Control (NC) > 0.150 OD450nm after blanking coefficient of variation > 30%	1. that the washing procedure and the washer settings are as validated in the pre qualification study; 2. that the proper washing solution has been used and the washer has been primed with it before use; 3. that no mistake has been done in the assay procedure (dispensation of positive control instead of negative control); 4. that no contamination of the negative control or of the wells where the control was dispensed has occurred due to positive samples, to spills or to the enzyme conjugate; 5. that micropipettes have not become contaminated with positive samples or with the enzyme conjugate 6. that the washer needles are not blocked or partially obstructed.
Calibrator S/Co < 2	1. that the procedure has been correctly performed; 2. that no mistake has occurred during its distribution (ex.: dispensation of negative control instead); 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the calibrator has occurred.
Positive Control < 1.500 OD450nm	1. that the procedure has been correctly performed; 2. that no mistake has occurred during the distribution of the control (dispensation of negative control instead of positive control); 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the positive control has occurred.

HBe antibody

Problem	Check
Blank well > 0.100 OD450nm	1. that the Chromogen/Substrate solution has not become contaminated during the assay
Negative Control (NC) < 1.000 OD450nm after blanking coefficient of variation > 10%	1. that the washing procedure and the washer settings are as validated in the pre qualification study; 2. that the proper washing solution has been used and the washer has been primed with it before use; 3. that no mistake has been done in the assay procedure (e.g.: dispensation of positive control instead of negative control; no dispensation of the Neutralizing Antigen; no dispensation of the Enzyme Conjugate); 4. that no contamination of the negative control or of the wells where the control was dispensed has occurred; 5. that micropipettes have not become contaminated with positive samples; 6. that the washer needles are not blocked or partially obstructed.

Calibrator OD450nm > NC/1.5	1. that the procedure has been correctly performed; 2. that no mistake has occurred during its distribution (ex.: dispensation of negative control instead; no dispensation of the Neutralizing Antigen; no dispensation of the Enzyme Conjugate); 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the calibrator has occurred.
Positive Control OD450nm > NC/10	1. that the procedure has been correctly performed; 2. that no mistake has occurred during the distribution of the control; 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the positive control has occurred.

If any of the above problems have occurred, report the problem to the supervisor for further actions.

Important note:
The analysis must be done proceeding as the reading step described in the section M, point 14.

P. CALCULATION OF THE CUT-OFF
The results are calculated by means of a cut-off value determined with the following formula:

HBeAg:

NC + 0.100 = Cut-Off (Co)

The value found for the test is used for the interpretation of results as described in the next paragraph.

HBeAb:

(NC + PC) / 3 = Cut-Off (Co)

***Important note**: When the calculation of results is performed by the operating system of an ELISA automated work station, ensure that the proper formulation is used to calculate the cut-off value and generate the correct interpretation of results.*

Q. INTERPRETATION OF RESULTS
Results are interpreted as follows:

HBeAg:

S/Co	Interpretation
< 0.9	Negative
0.9 - 1.1	Equivocal
> 1.1	Positive

HBeAb:

Co/S	Interpretation
< 0.9	Negative
0.9 - 1.1	Equivocal
> 1.1	Positive

Note:
S = OD450nm/620-630nm of the sample
Co = cut-off value

An example of calculation for HBeAg assay is reported below (data obtained proceeding as the the reading step described in the section M, point 14):

The following data must not be used instead or real figures obtained by the user.

Negative Control: 0.020 – 0.030 – 0.025 OD450nm
Mean Value: 0.025 OD450nm
Lower than 0.150 – Accepted

Positive Control: 2.489 OD450nm
Higher than 1.500 – Accepted
Cut-Off = 0.025+0.100 = 0.125
Calibrator: 0.520 - 0.540 OD450nm
Mean value: 0.530 OD450nm S/Co = 4.2
S/Co higher than 2.0 – Accepted

Sample 1: 0.030 OD450nm
Sample 2: 1.800 OD450nm
Sample 1 S/Co < 0.9 = negative
Sample 2 S/Co > 1.1 = positive

An example of calculation for HBeAb is reported below (data obtained proceeding as the the reading step described in the section M, point 14):

The following data must not be used instead or real figures obtained by the user.

Negative Control: 2.100 – 2.200 – 2.000 OD450nm
Mean Value: 2.100 OD450nm
Higher than 1.000 – Accepted

Positive Control: 0.100 OD450nm
Lower than NC/10 – Accepted

Cut-Off = (2.100 + 0.100) / 3 = 0.733
Calibrator: 0.720-0.760 OD450nm
Mean value: 0.740 OD450nm
OD450nm < NC/1.5 – Accepted

Sample 1: 0.020 OD450nm
Sample 2: 1.900 OD450nm
Sample 1 Co/S > 1.1 positive
Sample 2 Co/S < 0.9 negative

Important notes:
1. *Interpretation of results should be done under the supervision of the laboratory director to reduce the risk of judgment errors and misinterpretations.*
2. *The Identification of the clinical status of a HBV patient (acute, chronic, asymptomatic hepatitis) has to be done on the basis also of the other markers of HBV infection (HBsAg, HBsAb, HBcAb, HBcIgM);*
3. *When test results are transmitted from the laboratory to another facility, attention must be paid to avoid erroneous data transfer.*
4. *Diagnosis of viral hepatitis infection has to be taken by and released to the patient by a suitably qualified medical doctor.*

R. PERFORMANCE CHARACTERISTICS

A) HBeAg

1. Limit of detection
The limit of detection of the assay has been calculated by means of the International Standard for HBeAg, supplied by Paul Erlich Institute (PEI).
The data obtained by examining the limit of detection on three lots is reported in the table below.

HBE.CE Lot ID	PEI U/ml HBeAg
0103	0.25
0103/2	0.25
0303	0.25

In addition the preparation Accurun # 51, produced by Boston Biomedica Inc., USA, has been tested, upon dilution in FCS. Results are reported for three lots of products.

BBI's Accurun 51 (S/Co)

HBE.CE Lot ID	1 x	2 x	4 x	8 x	16x
0103	**4.1**	**1.6**	0.9	0.6	0.4
0103/2	**4.1**	**1.7**	0.9	0.6	0.4
0303	**4.0**	**1.6**	0.9	0.5	0.4

2. Diagnostic Sensitivity:
The diagnostic sensitivity has been tested on panels of samples classified positive by a US FDA approved kit.
Positive samples were collected from different HBV pathologies (acute, chronic) bearing HBeAg reactivity.
An overall value > 98% has been found in the study conducted on a total number of more than 200 samples.
Moreover the Panel of Seroconversion code PHM 935B, produced by BBI, was examined.
Data are reported below and compared with those reported by BBI for two other commercial products.

Sample ID	HBE.CE S/Co	Abbott EIA S/Co	Sorin EIA S/Co
21	5.4	4.5	6.3
22	3.7	4.3	5.4
23	1.9	3.2	3.1
24	1.1	2.4	1.5
25	1.0	2.1	1.2
26	0.6	1.7	0.7
27	0.2	0.8	0.3
28	0.2	0.6	0.2
29	0.2	0.4	0.2
30	0.2	0.3	0.2
31	0.1	0.3	0.2
32	0.1	0.3	0.2

Finally the Performance Panel code PHJ 201, produced by BBI, was tested. Data are reported below and compared with those reported by BBI for an other commercial product.

Member	PEI U/ml	HBE.CE	Sorin EIA
1	3	3.3	7.0
2	6	17.5	21.9
3	26	30.1	37.1
4	31	29.4	23.5
5	1	1.1	2.2
6	2	2.3	6.9
7	35	30.1	24.6
8	38	29.2	31.9
9	4	16.6	10.8
10	-	0.3	0.2
11	1	3.4	3.6
12	< 1	0.2	1.2
13	< 1	0.9	1.4
14	-	0.2	0.2
15	-	0.4	0.1
16	-	0.5	0.1
17	-	0.3	0.2
18	-	0.2	0.2
19	-	0.2	0.1
20	-	0.2	0.1
21	-	0.3	1.0
22	-	0.3	0.1
23	-	0.4	0.1
24	-	0.2	0.2
25	-	0.3	0.2

3. Diagnostic Specificity:
The diagnostic specificity has been determined on panels of negative samples from normal individuals and blood donors, classified negative with a FDA approved kit.
Both plasma, derived with different standard techniques of preparation (citrate, EDTA and heparin), and sera have been used to determine the specificity.
No false reactivity due to the method of specimen preparation has been observed.
Frozen specimens have also been tested to check whether this interferes with the performance of the test. No interference was observed on clean and particle free samples.
Samples derived from patients with different viral (HCV and HAV) and non viral pathologies of the liver that may interfere with the test were examined.
No cross reaction were observed.
The Performance Evaluation study conducted in a qualified external reference center on more than 500 samples has provided a value > 98% .

4. Precision
It has been calculated on two samples examined in 16 replicate in three different runs on three lots.
The values found were as follows:

HBE.CE: lot # 0103

Negative Control (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.030	0.027	0.032	0.029
Std.Deviation	0.002	0.002	0.003	0.002
CV %	7.4	8.2	7.9	7.8

PEI 1 U/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.569	0.559	0.575	0.568
Std.Deviation	0.027	0.029	0.028	0.028
CV %	4.7	5.3	4.9	4.9
S/Co	4.4	4.4	4.4	4.4

HBE.CE: lot # 0103/2

Negative Control (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.033	0.031	0.030	0.032
Std.Deviation	0.003	0.003	0.002	0.003
CV %	7.9	8.5	7.4	8.0

PEI 1 U/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.565	0.573	0.568	0.569
Std.Deviation	0.026	0.025	0.024	0.025
CV %	4.7	4.3	4.2	4.4
S/Co	4.2	4.4	4.4	4.3

HBE.CE: lot # 0303

Negative Control (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.029	0.034	0.038	0.034
Std.Deviation	0.003	0.003	0.004	0.003
CV %	9.7	9.8	9.2	9.6

PEI 1 U/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.579	0.573	0.564	0.572
Std.Deviation	0.023	0.028	0.025	0.025
CV %	4.1	4.8	4.5	4.5
S/Co	4.5	4.3	4.1	4.3

B) HBe Antibody

1. Limit of detection

The limit of detection of the assay has been calculated by means of the International Standard for HBeAb, supplied by Paul Erlich Institute (PEI).

The data obtained by examining the limit of detection on three lots is reported in the table below.

HBE.CE Lot ID	PEI U/ml HBeAb
0103	0.25
0103/2	0.25
0303	0.25

In addition the preparation Accurun # 52, produced by Boston Biomedica Inc., USA, has been tested, upon dilution in FCS. Results are reported for three lots of products.

Accurun 52 (Co/S)

HBE.CE Lot ID	1 x	2 x	4 x	8 x	16x
0103	1.0	0.8	0.6	0.4	0.4
0103/2	1.0	0.8	0.6	0.5	0.4
0303	1.0	0.8	0.6	0.4	0.4

2. Diagnostic sensitivity:

The diagnostic sensitivity has been tested on panels of samples classified positive for HBeAb by a US FDA approved kit.

Positive samples were collected from different HBV pathologies bearing anti HBeAg antibody reactivity.

An overall value > 98% has been found in the study conducted on a total number of more than 200 samples.

Moreover the Panel of Seroconversion code PHM 935B, produced by BBI, was examined.

Data are reported below and compared with those reported by BBI for two other commercial products.

Sample ID	HBE.CE Co/S	Abbott EIA Co/S	Sorin EIA Co/S
21	0.4	0.4	0.5
22	0.4	0.5	0.6
23	0.4	0.6	0.5
24	0.4	0.5	0.6
25	0.4	0.6	0.5
26	0.5	0.6	0.6
27	0.6	0.8	0.7
28	0.7	0.9	0.7
29	0.6	0.9	0.7
30	0.8	1.0	0.9
31	1.0	1.3	1.1
32	1.0	1.2	1.0

Finally the Performance Panel code PHJ 201, produced by BBI, was tested. Data are reported below and compared with those reported by BBI for another commercial product.

Member	PEI U/ml	HBE.CE	Sorin EIA
1	-	0.3	0.5
2	-	0.2	0.5
3	-	0.2	0.5
4	-	0.2	0.5
5	-	0.3	0.6
6	-	0.3	0.6
7	-	0.2	0.4
8	-	0.2	0.4
9	-	0.2	0.5
10	-	1.9	0.6
11	-	0.3	0.5
12	-	0.4	0.9
13	2	4.4	9.1
14	1	3.8	2.9
15	< 1	1.0	1.5
16	> 50	4.3	120.9
17	< 1	1.0	1.0
18	5	5.6	21.8
19	1	2.7	6.4
20	11	5.0	47.3
21	2	1.9	10.0
22	26	28.1	90.7
23	-	0.3	0.5
24	< 1	0.8	1.3
25	50	28.1	167.4

3. Diagnostic specificity:

The clinical specificity has been determined as described before for HBeAg.

The Performance Evaluation study conducted in a qualified external reference center on more than 500 samples has provided a value > 98% .

4. Precision:

It has been calculated on two samples examined in 16 replicate in three different runs on three lots.

The values found were as follows:

HBE.CE: lot # 0103

Negative Control (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	2.484	2.420	2.471	2.458
Std.Deviation	0.129	0.160	0.142	0.144
CV %	5.2	6.6	5.7	5.9

PEI 0.25 U/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.867	0.800	0.878	0.848
Std.Deviation	0.043	0.060	0.050	0.051
CV %	5.0	7.5	5.7	6.1
Co/S	1.0	1.0	1.0	1.0

HBE.CE: lot # 0103/2

Negative Control (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	2.316	2.361	2.413	2.363
Std.Deviation	0.127	0.144	0.146	0.139
CV %	5.5	6.1	6.0	5.9

PEI 0.25 U/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.767	0.793	0.785	0.781
Std.Deviation	0.041	0.050	0.046	0.046
CV %	5.4	6.3	5.8	5.8
Co/S	1.0	1.0	1.0	1.0

HBE.CE: lot #0303

Negative Control (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	2.334	2.415	2.437	2.395
Std.Deviation	0.146	0.155	0.158	0.153
CV %	6.3	6.4	6.5	6.4

PEI 0.25 U/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.850	0.867	0.876	0.864
Std.Deviation	0.052	0.051	0.048	0.050
CV %	6.1	5.9	5.5	5.8
Co/S	0.9	1.0	1.0	1.0

Important note:
The performance data have been obtained proceeding as the reading step described in the section M, point 14.

S. LIMITATIONS

Frozen samples containing fibrin particles or aggregates may generate false positive results.
Bacterial contamination or heat inactivation of the specimen may affect the absorbance values of the samples with consequent alteration of the level of the analyte.
This test is suitable only for testing single samples and not pooled ones.
Diagnosis of an infectious disease should not be established on the basis of a single test result. The patient's clinical history, symptomatology, as well as other diagnostic data should be considered.

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All the IVD Products manufactured by the company are under the control of a certified Quality Management System approved by an EC Notified Body. Each lot is submitted to a quality control and released into the market only if conforming with the EC technical specifications and acceptance criteria.

Manufacturer:
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CE
0318

Monobind Inc.
Lake Forest, CA 92630, USA

AccuBind
ELISA Microwells

Dehydroepiandrosterone Sulfate (DHEA-S) Test System

Product Code: 5125-300

1.0 INTRODUCTION

Intended Use: The Quantitative Determination of Dehydroepiandrosterone Sulfate Concentration in Human Serum or Plasma by a Microplate Enzyme Immunoassay, Colorimetric

2.0 SUMMARY AND EXPLANATION OF THE TEST

Dehydroepiandrosterone sulfate (DHEA-S) is the major C19 steroid secreted by the adrenal cortex, and is a precursor in testosterone and estrogen biosynthesis. DHEA-S, the sulfate ester of DHEA, is derived from sulfated precursors and by enzymatic conversion of DHEA in adrenal and extradrenal tissues. Due to the presence of a 17-oxo [rather than hydroxyl] group, DHEA-S possesses relatively weak androgenic activity, which for unsulfated DHEA has been estimated at ~10% that of testosterone.[1] However, the bioactivity of DHEA-S may be increased by its relatively high serum concentrations, approximately 100 to 1000-fold higher than DHEA or testosterone, and its weak affinity for sex-hormone binding globulin.[2]

The physiologic role of DHEA-S is not well-defined. Serum levels are relatively high in the fetus and neonate, low during childhood, and increase during puberty.[3,4] Increased levels of DHEA-S during adrenarche may contribute to the development of secondary sexual hair. DHEA-S levels show a progressive decline after the third decade of life.[5] Unlike DHEA, DHEA-S levels do not show significant diurnal variation and little day-to-day variation. DHEA-S levels are not responsive to acute corticotropin administration,[4] and do not vary significantly during the normal menstrual cycle.[2] This may be due to the slower metabolic clearance rate of DHEA-S as compared to DHEA.[6]

Measurement of serum DHEA-S is a useful marker of adrenal androgen synthesis. Abnormally low levels have been reported in hypoadrenalism,[3] while elevated levels occur in several conditions; including virilizing adrenal adenoma and carcinoma,[7] 21-hydroxylase and 3β-hydroxysteroid dehydrogenase deficiencies[2,8] and some cases of female hirsutism.[2] Since very little DHEA-S is produced by the gonads,[2,3] measurement of DHEA-S may aid in the localization of the androgen source in virilizing conditions. Methods for measurement of DHEA-S include gas-liquid chromatography, double-isotope derivative techniques, competitive protein-binding assays, and radioimmunoassay. Although significant cross-reactivity occurs with DHEA, androstenedione and androsterone, the relative concentrations of these competing substances in most normal and pathologic samples predicts a minimal effect on assay performance.

The Monobind DHEA-S ELISA Kit uses a specific anti-DHEA-S antibody, and does not require prior sample extraction of serum or plasma. Cross-reactivity to other naturally occurring and structurally related steroids is low. The employment of several serum references of known DHEA-S concentration permits construction of a graph of activity and concentration. From comparison to the dose response curve, an unknown specimen's activity can be correlated with DHEA-S concentration.

3.0 PRINCIPLE

Competitive Enzyme Immunoassay (TYPE 7):
The essential reagents required for an enzyme immunoassay include antibody, enzyme-antigen conjugate and native antigen. Upon mixing biotinylated antibody, enzyme-antigen conjugate and a serum containing the native antigen, a competition reaction results between the native antigen and the enzyme-antigen conjugate for a limited number of antibody binding sites. The interaction is illustrated by the following equation:

$$^{Enz}Ag + Ag + Ab_{Btn} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} AgAb_{Btn} + {}^{Enz}AgAb_{Btn}$$

Ab_{Btn} = Biotinylated x-DHEA-S IgG Antibody (Constant Quantity)
Ag = Native Antigen (Variable Quantity)
^{Enz}Ag = Enzyme-antigen Conjugate (Constant Quantity)
$AgAb_{Btn}$ = Antigen-Antibody Complex
$^{Enz}Ag\,Ab_{Btn}$ = Enzyme-antigen Conjugate -Antibody Complex
k_a = Rate Constant of Association
k_{-a} = Rate Constant of Disassociation
$K = k_a / k_{-a}$ = Equilibrium Constant

A simultaneous reaction between the biotin attached to the antibody and the streptavidin immobilized on the microwell occurs. This effects the separation of the antibody bound fraction after decantation or aspiration.

$AgAb_{Btn} + {}^{Enz}AgAb_{Btn} + \underline{\text{Streptavidin}}_{CW} \Rightarrow \underline{\text{immobilized complex}}$

$\underline{\text{Streptavidin}}_{CW}$ = Streptavidin immobilized on well
Immobilized complex = sandwich complex bound to the solid surface

The enzyme activity in the antibody bound fraction is inversely proportional to the native antigen concentration. By utilizing several different serum references of known antigen concentration, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

4.0 REAGENTS

Materials Provided:

A. DHEA-S Calibrators – 1ml/vial - Icons A-F
Six (6) vials of serum reference for DHEA-S at concentrations of 0 (**A**), 0.2 (**B**), 1.0 (**C**), 2.0 (**D**), 4.0 (**E**) and 8.0 (**F**) in µg/ml. Store at 2-8°C. A preservative has been added. The calibrators can be expressed in molar concentrations (nM/L) by using 2.71 as a conversion factor.
For example: 1µg/ml x 2.71 = 2.71 µM/L

B. DHEA-S Enzyme Reagent – 6.0 ml/vial Ⓔ
One (1) vial of DHEA-S (Analog)-horseradish peroxides (HRP) conjugate in a protein-stabilizing matrix with red dye. Store at 2-8°C.

C. DHEA-S Biotin Reagent – 6.0 ml - Icon ▽
One (1) bottle of reagent contains anti-DHEA-S biotinylated purified rabbit IgG conjugate in buffer, blue dye and preservative. Store at 2-8°C.

D. Streptavidin Coated Plate – 96 wells –Icon ⇓
One 96-well microplate coated with 1.0 µg/ml streptavidin and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

E. Wash Solution Concentrate – 20ml/vial - Icon
One (1) vial contains a surfactant in buffered saline. A preservative has been added. Store at 2-8°C.

F. Substrate A – 7ml/vial - Icon S[A]
One (1) vial contains tetramethylbenzidine (TMB) in buffer. Store at 2-8°C.

G. Substrate B – 7ml/vial - Icon S[B]
One (1) vial contains hydrogen peroxide (H_2O_2) in buffer. Store at 2-8°C.

H. Stop Solution -- 8ml/vial - Icon STOP
One (1) vial contains a strong acid (1N HCl). Store at 2-8°C.

I. Product Instructions

Note 1: Do not use reagents beyond the kit expiration date.
Note 2: Avoid extended exposure to heat and light. **Opened reagents are stable for sixty (60) days when stored at 2-8°C. Kit and component stability are identified on the label.**
Note 3: Above reagents are for a single 96-well microplate.

4.1 Required But Not Provided:
1. Pipette capable of delivering 0.010ml (10µl) and 0.050ml (50µl) with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100ml (100µl) and 0.350ml (350µl) volumes with a precision of better than 1.5%.
3. Adjustable volume (200-1000µl) dispenser(s) for conjugate.
4. Microplate washer or a squeeze bottle (optional).
5. Microplate Reader with 450nm and 620nm wavelength absorbance capability.
6. Absorbent Paper for blotting the microplate wells.
7. Plastic wrap or microplate cover for incubation steps.
8. Vacuum aspirator (optional) for wash steps.
9. Timer.
10. Quality control materials.

5.0 PRECAUTIONS

For In Vitro Diagnostic Use
Not for Internal or External Use in Humans or Animals

All products that contain human serum have been found to be non-reactive for Hepatitis B Surface Antigen, HIV 1&2 and HCV Antibodies by FDA required tests. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Edition, 1988, HHS Publication No. (CDC) 88-8395.

Safe Disposal of kit components must be according to local regulatory and statutory requirement.

6.0 SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood serum or heparanised plasma in type, and taken with the usual precautions in the collection of venipuncture samples. For accurate comparison to establish normal values, a fasting morning serum sample should be obtained. The blood should be collected in a redtop veni-puncture tube with or without additives or anti-coagulants (for serum) or evacuated tube(s) containing EDTA or heparin (for plasma). Allow the blood to clot for serum samples. Centrifuge the specimen to separate the serum or plasma from the cells.

In patients receiving therapy with high biotin doses (i.e. >5mg/day), no sample should be taken until at least 8 hours after the last biotin administration, preferably overnight to ensure fasting sample.

Samples may be refrigerated at 2-8°C for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20°C for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.020ml (20µl) of the specimen is required.

7.0 QUALITY CONTROL

Each laboratory should assay controls at levels in the low, normal and high range for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. The individual laboratory should set acceptable assay performance limits. In addition, maximum absorbance should be consistent with past experience. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

8.0 REAGENT PREPARATION

1. **Wash Buffer**
Dilute contents of wash solution to 1000ml with distilled or deionized water in a suitable storage container. Diluted buffer can be stored at 2-30°C for up to 60 days.

2. **Working Substrate Solution - Stable for 1 year**
Pour the contents of the amber vial labeled Solution 'A' into the clear vial labeled Solution 'B'. Place the yellow cap on the clear vial for easy identification. Mix and label accordingly. Store at 2 - 8°C.

Note1: Do not use the working substrate if it looks blue.
Note 2: Do not use reagents that are contaminated or have bacteria growth.

9.0 TEST PROCEDURE

Before proceeding with the assay, bring all reagents, serum reference calibrators and controls to room temperature (20-27°C).
*****Test Procedure should be performed by a skilled individual or trained professional*****

1. Format the microplates' wells for each serum reference calibrator, control and patient specimen to be assayed in duplicate. **Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.**
2. Pipette 0.010 ml (10 µL) of the appropriate serum reference calibrator, control or specimen into the assigned well.
3. Add 0.050 ml (50µl) of the DHEA-S Enzyme Reagent to all wells.
4. Swirl the microplate gently for 20-30 seconds to mix.
5. Add 0.050 ml (50µl) of Anti- DHEA-S Biotin Reagent to all wells.
6. Swirl the microplate gently for 20-30 seconds to mix.
7. Cover and incubate for 30 minutes at room temperature.
8. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.
9. Add 0.350ml (350µl) of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.**
10. Add 0.100 ml (100µl) of working substrate solution to all wells (see Reagent Preparation Section). **Always add reagents in the same order to minimize reaction time differences between wells.**

DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION

11. Incubate at room temperature for fifteen (15) minutes.
12. Add 0.050ml (50µl) of stop solution to each well and gently mix for 15-20 seconds. **Always add reagents in the same order to minimize reaction time differences between wells.**
13. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm.. **The results should be read within thirty (30) minutes of adding the stop solution.**

Note: Dilute the samples suspected of concentrations higher than 8.0 ug/ml 1:5 and 1:10 with DHEA-S '0' µg/ml calibrator or patient serum pools with a known low value for DHEA-S.

10.0 CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of DHEA-S in unknown specimens.
1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the absorbance for each duplicate serum reference versus the corresponding DHEA-S concentration in ug/ml on linear graph paper (do not average the duplicates of the serum references before plotting).
3. Connect the points with a best-fit curve.
4. To determine the concentration of DHEA-S for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in ug/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance in the patient sample (1.078) intersects the dose response curve at (1.21 µg/ml) DHEA-S concentration (See Figure 1).

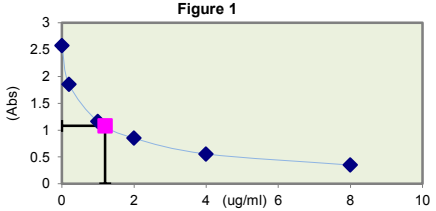
Note: Computer data reduction software designed for ELISA assays may also be used for the data reduction. If such

software is utilized, the validation of the software should be ascertained.

EXAMPLE 1

Sample I.D.	Well Number	Abs (A)	Mean Abs (B)	Value (µg/ml)
Cal A	A1	2.562	2.572	0.0
	B1	2.582		
Cal B	C1	1.865	1.847	0.2
	D1	1.829		
Cal C	E1	1.186	1.163	1.0
	F1	1.140		
Cal D	G1	0.855	0.850	2.0
	H1	0.845		
Cal E	A2	0.555	0.556	4.0
	B2	0.557		
Cal F	C2	0.355	0.349	8.0
	D2	0.344		
Cont 1	G2	1.394	1.387	0.62
	H2	1.380		
Pat# 1	A3	1.065	1.078	**1.21**
	B3	1.091		

Figure 1



*The represented in Example 1 and Firgure 1 is for illustration only and **should NOT** be used in lieu of a dose response curve prepared with each assay.

11.0 Q.C. PARAMETERS

In order for the assay results to be considered valid the following criteria should be met:

1. The absorbance (OD) of calibrator 0 ug/ml should be ≥ 1.3
2. Four out of six quality control pools should be within the established ranges.

12.0 RISK ANALYSIS

The MSDS and Risk Analysis Form for this product are available on request from Monobind Inc.

12.1 Assay Performance

1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. The addition of substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the substrate and stop solution should be added in the same sequence to eliminate any time-deviation during reaction.
6. Plate readers measure vertically. Do not touch the bottom of the wells.
7. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
8. Use components from the same lot. No intermixing of reagents from different batches.
9. Patient specimens with DHEA-S concentrations above 8.0 µg/mL may be diluted (1/5, 1/10 or higher) with DHEA-S '0' calibrator and re-assayed. The sample's concentration is obtained by multiplying the result by the dilution factor.
10. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from Monobind' IFU may yield inaccurate results.
11. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.
12. It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.
13. Risk Analysis- as required by CE Mark IVD Directive 98/79/EC - for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com.

12.2 Interpretation

1. ***Measurements and interpretation of results must be performed by a skilled individual or trained professional.***
2. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.
3. The reagents for the test system have been formulated to eliminate maximal interference; however, potential interaction between rare serum specimens and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for all kinds of immunoassays (Boscato LM, Stuart MC. 'Heterophilic antibodies: a problem for all immunoassays' Clin. Chem. 1988:3427-33). For diagnostic purposes, the results from this assay should be in combination with clinical examination, patient history and all other clinical findings.
4. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
5. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, Monobind shall have no liability.
6. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.
7. Clinically, **a DHEA-S value alone is not of diagnostic value** and should only be used in conjunction with other clinical manifestations (observations) and diagnostic procedures.

13.0 EXPECTED RANGES OF VALUES

In agreement with established reference intervals for a "normal" adult population, the expected ranges for the DHEA-S AccuBind® ELISA Test System are detailed in Table 1.

TABLE I
Expected Values for the DHEA-S Test System

POPULATION	RANGE (µg/ml)
Male	0.06 – 4.58
Female	0.03 – 5.88

It is important to keep in mind that establishment of a range of values which can be expected to be found by a given method for a population of "normal" persons is dependent upon a multiplicity of factors: the specificity of the method, the population tested and the precision of the method in the hands of the analyst. For these reasons each laboratory should depend upon the range of expected values established by the manufacturer only until an in-house range can be determined by the analysts using the method with a population indigenous to the area in which the laboratory is located.

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

The within and between assay precision of the DHEA-S AccuBind® ELISA Test System were determined by analyses on three different levels of pool control sera. The number, mean values, standard deviation and coefficient of variation for each of these control sera are presented in Table 2 and Table 3.

TABLE 2
Within Assay Precision (Values in µg/ml)

Sample	N	X	σ	C.V.
Low	16	0.66	0.06	9.8%
Normal	16	1.14	0.05	4.9%
High	16	4.84	0.21	4.3%

TABLE 3
Between Assay Precision (Values in µg/ml)

Sample	N	X	σ	C.V.
Low	10	0.61	0.06	9.5%
Normal	10	1.36	0.04	3.1%
High	10	4.73	0.16	3.4%

*As measured in ten experiments in duplicate over a ten day period.

14.2 Sensitivity

The DHEA-S AccuBind® ELISA Test System has a sensitivity of 0.042 ug/ml. The sensitivity was ascertained by determining the variability of the 0 ug/ml serum calibrator and using the 2σ (95% certainty) statistic to calculate the minimum dose.

14.3 Accuracy

The DHEA-S AccuBind® ELISA Test System was compared with a chemiluminescence immunoassay method. Biological specimens from low, normal and relatively high DHEA-S level populations were used (The values ranged from 0.2 ug/ml – 7.7 ug/ml). The total number of such specimens was 77. The least square regression equation and the correlation coefficient were computed for this DHEA-S EIA in comparison with the reference method. The data obtained is displayed in Table 4.

TABLE 4

Method	Mean (x)	Least Square Regression Analysis	Correlation Coefficient
Monobind (y)	1.12	y= 0.1448+0.986x)	0.983
Reference (X)	1.18		

Only slight amounts of bias between this method and the reference method are indicated by the closeness of the mean values. The least square regression equation and correlation coefficient indicates excellent method agreement.

14.4 Specificity

The % cross reactivity of the DHEA-S antibody to selected substances was evaluated by adding the interfering substance to a serum matrix at various concentrations. The cross-reactivity was calculated by deriving a ratio between dose of interfering substance to dose of DHEA-S needed to displace the same amount of labeled analog.

Substance	Cross Reactivity
DHEA-S	1.0000
DHEA	0.0004
Androstenedione	0.0003
Dihydotestosterone	0.0008
Cortisone	<0.0001
Corticosterone	<0.0001
Cortisol	0.0004
Spirolactone	<0.0001
Estriol	<0.0001
Estradiol	<0.0001
Estrone	<0.0001
Testosterone	<0.0001

15.0 REFERENCES

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Revision: 5 Date: 2019-Jul-16 DCO: 1353
MP5125 Product Code: 5125-*300*

Size		96(A)	192(B)
Reagent (fill)	A)	1ml set	1ml set
	B)	1 (6ml)	2 (6ml)
	C)	1 (6ml)	2 (6ml)
	D)	1 plate	2 plates
	E)	1 (20ml)	1 (20ml)
	F)	1 (7ml)	2 (7ml)
	G)	1 (7ml)	2 (7ml)
	H)	1 (8ml)	2 (8ml)

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www.cepartner4u.eu

Please visit our website to learn more about our products and services.

Glossary of Symbols
(EN 980/ISO 15223)

IVD — In Vitro - Diagnostic Medical Device
2° 8° — Temperature Limitation Storage Condition (2-8°C)
Consult Instructions for Use
REF — Catalogue Number
Σ — Contains Sufficient Test for Σ
LOT — Batch Code
Used By (Expiration Day)
Date of Manufacturer
Manufacturer
EC REP — Authorized Rep in European Country
CE — European Conformity

Monobind Inc.
Lake Forest, CA 92630, USA

AccuBind
ELISA Microwells

Estradiol (E2) Test System
Product Code: 4925-300

1.0 INTRODUCTION

Intended Use: The Quantitative Determination of Estradiol Concentration in Human Serum or Plasma by a Microplate Enzyme Immunoassay, Colorimetric

2.0 SUMMARY AND EXPLANATION OF THE TEST

Measurement of estradiol in serum or plasma is considered to be the most reliable way to assess its rate of production.

Estradiol (17β-estradiol) is a steroid hormone (molecular weight of 272.3 daltons), which circulates predominantly protein-bound. In addition to estradiol, other natural steroidal estrogens include estrone, estriol and their metabolites. Natural estrogens are hormones secreted principally by the ovarian follicles and also by the adrenals, corpus luteum, and placenta and, in males, by the testes. Exogenous estrogens (natural or synthetic) elicit, to varying degrees, all the pharmacologic responses usually produced by endogenous estrogens.

Estrogenic hormones are secreted at varying rates during the menstrual cycle throughout the period of ovarian activity. During pregnancy, the placenta becomes the main source of estrogens. At menopause, ovarian secretion of estrogens declines at varying rates. The gonadotropins of the anterior pituitary regulate secretion of the ovarian hormones, estradiol and progesterone; hypothalamic control of pituitary gonadotropin production is in turn regulated by plasma concentrations of the estrogens and progesterone. This complex feedback system results in the cyclic phenomenon of ovulation and menstruation.

Estradiol determinations have proved of value in a variety of contexts, including the investigation of precocious puberty in girls and gynecomastia in men. Its principal uses have been in the differential diagnosis of amenorrhea and in the monitoring of ovulation induction.

This kit uses a specific anti-estradiol antibody, and does not require prior sample extraction of serum or plasma. Cross-reactivity to other naturally occurring and structurally related steroids is low.

The employment of several serum references of known estradiol concentration permits construction of a graph of activity and concentration. From comparison to the dose response curve, an unknown specimen's activity can be correlated with estradiol concentration.

3.0 PRINCIPLE

Delayed Competitive Enzyme Immunoassay (TYPE 9):
The essential reagents required for an enzyme immunoassay include antibody, enzyme-antigen conjugate and native antigen. Upon mixing the biotinylated antibody with a serum containing the antigen, a reaction results between the antigen and the antibody. The interaction is illustrated by the following equation:

$$Ag + Ab_{Btn} \rightleftharpoons AgAb_{Btn}$$

Ab_{Btn} = Biotinylated antibody
Ag = Antigen (Variable Quantity) $AgAb_{Btn}$ = Immune Complex

After a short incubation, the enzyme conjugate is added (This delayed addition permits an increase in sensitivity for low concentration samples). Upon the addition of the enzyme conjugate, competition reaction results between the enzyme analog and the antigen in the sample for a limited number of antibody binging sites (not consumed in the first incubation).

$$^{Enz}Ag + Ag + rAb_{Btn} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} AgAb_{Btn} + {}^{Enz}AgAb_{Btn}$$

^{Enz}Ag = Enzyme-antigen Conjugate (Constant Quantity)
$^{Enz}AgAb_{Btn}$ = Enzyme-antigen Conjugate -Antibody Complex
rAb_{Btn} = Biotinylated antibody not reacted in first incubation
k_a = Rate Constant of Association
k_{-a} = Rate Constant of Disassociation
$K = k_a / k_{-a}$ = Equilibrium Constant

A simultaneous reaction between the biotin attached to the antibody and the streptavidin immobilized on the microwell occurs. This effects the separation of the antibody bound fraction after decantation or aspiration.

$AgAb_{Btn} + {}^{Enz}AgAb_{Btn} + \underline{Streptavidin}_{CW} \Rightarrow$ <u>immobilized complex</u>
$\underline{Streptavidin}_{CW}$ = Streptavidin immobilized on well
<u>Immobilized complex</u> = sandwich complex bound to the solid surface

The enzyme activity in the antibody bound fraction is inversely proportional to the native antigen concentration. By utilizing several different serum references of known antigen concentration, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

4.0 REAGENTS

Materials Provided

A. Estradiol Calibrators – 1ml/vial - Icons A-G
Seven (7) vials of serum reference for estradiol at concentrations of 0 (**A**), 20 (**B**), 100 (**C**), 250 (**D**), 500 (**E**), 1500 (**F**) and 3000 (**G**) in pg/ml. Store at 2-8°C. A preservative has been added. The calibrators can be expressed in molar concentrations (pM/L) by multiplying by 3.67.
For example: 1pg/ml x 3.67= 3.67 pM/L

B. Estradiol Enzyme Reagent – 6.0 ml/vial
One (1) vial of Estradiol (Analog)-horseradish peroxides (HRP) conjugate in a protein-stabilizing matrix red with dye. Store at 2-8°C.

C. Estradiol Biotin Reagent – 6.0 ml - Icon ▽
One (1) bottle of reagent contains anti-estradiol biotinylated purified rabbit IgG conjugate in buffer, green dye and preservative. Store at 2-8°C.

D. Streptavidin Coated Plate – 96 wells –Icon ⇓
One 96-well microplate coated with 1.0 µg/ml streptavidin and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

E. Wash Solution Concentrate – 20ml/vial - Icon
One (1) vial contains a surfactant in buffered saline. A preservative has been added. Store at 2-8°C.

F. Substrate Reagent – 12ml/vial - Icon S^N
One (1) vial contains tetramethylbenzidine (TMB) and hydrogen peroxide (H_2O_2) in buffer. Store at 2-8°C.

G. Stop Solution – 8ml/vial - Icon
One (1) vial contains a strong acid (0.5M H_2SO_4). Store at 2-8°C.

H. Product Instructions

Note 1: Do not use reagents beyond the kit expiration date.
Note 2: Avoid extended exposure to heat and light. **Opened reagents are stable for sixty (60) days when stored at 2-8°C. Kit and component stability are identified on label.**
Note 3: Above reagents are for a single 96-well microplate.

4.1 Required But Not Provided:
1. Pipette capable of delivering 0.025ml (25µl) and 0.050ml (50µl) with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100ml (100µl) and 0.350ml (350µl) volumes with a precision of better than 1.5%.
3. Microplate washer or a squeeze bottle (optional).
4. Absorbent Paper for blotting the microplate wells.
5. Microplate Reader with 450nm and 620nm wavelength absorbance capability.
6. Plastic wrap or microplate cover for incubation steps.
7. Vacuum aspirator (optional) for wash steps.
8. Timer.
9. Quality control materials.

5.0 PRECAUTIONS

For In Vitro Diagnostic Use
Not for Internal or External Use in Humans or Animals

All products that contain human serum have been found to be non-reactive for Hepatitis B Surface Antigen, HIV 1&2 and HCV Antibodies by FDA required tests. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Edition, 1988, HHS Publication No. (CDC) 88-8395.

Safe Disposal of kit components must be according to local regulatory and statutory requirement.

6.0 SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood, serum or heparanised plasma in type, and taken with the usual precautions in the collection of venipuncture samples. For accurate comparison to establish normal values, a fasting morning serum sample should be obtained. The blood should be collected in a redtop (with or without gel additives) venipuncture tube(s) or for plasma use evacuated tube(s) containing heparin.. Allow the blood to clot for serum samples. Centrifuge the specimen to separate the serum or plasma from the cells.

In patients receiving therapy with high biotin doses (i.e. >5mg/day), no sample should be taken until at least 8 hours after the last biotin administration, preferably overnight to ensure fasting sample.

Samples may be refrigerated at 2-8oC for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20oC for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.050ml of the specimen is required.

7.0 QUALITY CONTROL

Each laboratory should assay controls at levels in the low, normal and high range for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. The individual laboratory should set acceptable assay performance limits. In addition, maximum absorbance should be consistent with past experience. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

8.0 REAGENT PREPARATION

1. **Wash Buffer**
Dilute contents of wash solution to 1000ml with distilled or deionized water in a suitable storage container. Diluted buffer can be stored at 2-30°C for up to 60 days.

Note: Do not use reagents that are contaminated or have bacteria growth.

9.0 TEST PROCEDURE

Before proceeding with the assay, bring all reagents, serum reference calibrators and controls to room temperature (20-27°C).

*****Test Procedure should be performed by a skilled individual or trained professional*****

1. Format the microplates' wells for each serum reference calibrator, control and patient specimen to be assayed in duplicate. **Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.**
2. Pipette 0.025 ml (25 µL) of the appropriate serum reference calibrator, control or specimen into the assigned well.
3. Add 0.050 ml (50µl) of the Estradiol Biotin Reagent to all wells.
4. Swirl the microplate gently for 20-30 seconds to mix.
5. Cover and incubate for 30 minutes at room temperature.
6. Add 0.050 ml (50µl) of Estradiol Enzyme Reagent to all wells. **Add directly on top the reagents dispensed in the wells**.
7. Swirl the microplate gently for 20-30 seconds to mix.
8. Cover and incubate for 90 minutes at room temperature.
9. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.
10. Add 0.350ml (350µl) of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.**
11. Add 0.100 ml (100µl) of substrate solution to all wells. **Always add reagents in the same order to minimize reaction time differences between wells.**

DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION

12. Incubate at room temperature for twenty (20) minutes.
13. Add 0.050ml (50µl) of stop solution to each well and gently mix for 15-20 seconds. **Always add reagents in the same order to minimize reaction time differences between wells.**
14. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm. **The results should be read within fifteen (15) minutes of adding the stop solution.**

Note: Dilute the samples suspected of concentrations higher than 3000pg/ml 1:5 and 1:10 with estradiol '0' pg/ml calibrator or male patient serum pools with a known low value for estradiol.

10.0 CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of estradiol in unknown specimens.
1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the absorbance for each duplicate serum reference versus the corresponding estradiol concentration in pg/ml on linear graph paper (do not average the duplicates of the serum references before plotting).
3. Connect the points with a best-fit curve.
4. To determine the concentration of estradiol for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in pg/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance (1.202) intersects the dose response curve at (160pg/ml) estradiol concentration (See Figure 1).

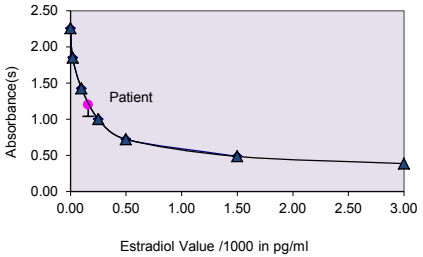
Note: Computer data reduction software designed for ELISA assay may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained.

EXAMPLE 1

Sample I.D.	Well Number	Abs (A)	Mean Abs (B)	Value (pg/ml)
Cal A	A1	2.268	2.256	0
	B1	2.244		
Cal B	C1	1.839	1.849	20
	D1	1.860		
Cal C	E1	1.409	1.426	100
	F1	1.443		
Cal D	G1	1.017	1.003	250
	H1	0.989		
Cal E	A2	0.698	0.723	500
	B2	0.748		
Cal F	C2	0.480	0.487	1500
	D2	0.493		
Cal G	E2	0.390	0.388	3000
	F2	0.385		
Pat# 1	G2	1.202	1.202	**160**
	H2	1.203		

*The data presented in Example 1 and Figure 1 is for illustration only and **should not** be used in lieu of a dose response curve prepared with each assay.

Figure 1



Note: Multiply the horizontal values by 1000 to convert into pg/ml.

11.0 Q.C. PARAMETERS

In order for the assay results to be considered valid the following criteria should be met:

1. The absorbance (OD) of calibrator 0 pg/ml should be ≥ 1.3
2. Four out of six quality control pools should be within the established ranges.

12.0 RISK ANALYSIS

The MSDS and Risk Analysis Form for this product are available on request from Monobind Inc.

12.1 Assay Performance

1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. The addition of substrate solution initiates a kinetic reaction, terminated by the addition of the stop solution. Therefore, the substrate and stop solution should be added in the same sequence to eliminate any time-deviation during reaction.
6. Plate readers measure vertically. Do not touch the bottom of the wells.
7. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
8. Use components from the same lot. No intermixing of reagents from different batches.
9. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from Monobind IFU may yield inaccurate results.
10. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper usage.
11. It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.
12. Risk Analysis- as required by CE Mark IVD Directive 98/79/EC - for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com.

12.2 Interpretation

1. ***Measurements and interpretation of results must be performed by a skilled individual or trained professional.***
2. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.
3. The reagents for the test system have been formulated to eliminate maximal interference; however, potential interaction between rare serum specimens and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for all kinds of immunoassays (Boscato LM, Stuart MC. 'Heterophilic antibodies: a problem for all immunoassays' Clin. Chem. 1988:3427-33). For diagnostic purposes, the results from this assay should be in combination with clinical examination, patient history and all other clinical findings.
4. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
5. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, Monobind shall have no liability.
6. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.

13.0 EXPECTED RANGES OF VALUES

In agreement with established reference intervals for a "normal" adult population and females during gestation the expected ranges for the Estradiol AccuBind® ELISA Test System are detailed in Table 1.

TABLE 1
Expected Values for the Estradiol Test System

	Median	Range
Females	-	-
Follicular Phase	48	9-175
Luteal Phase	103	44-196
Periovulatory	209	107-281
Treated Menopausal	122	42-289
Untreated Menopausal	7.3	ND-20
Oral Contraceptives	13	ND-103
Males	19	4-94

During pregnancy the Estradiol serum levels rise rapidly till the end of third trimester.[15]

It is important to keep in mind that establishment of a range of values which can be expected to be found by a given method for a population of "normal" persons is dependent upon a multiplicity of factors: the specificity of the method, the population tested and the precision of the method in the hands of the analyst. For these reasons each laboratory should depend upon the range of expected values established by the manufacturer only until an in-house range can be determined by the analysts using the method with a population indigenous to the area in which the laboratory is located.

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

The within and between assay precision of the estradiol AccuBind® Microplate ELISA Test System were determined by analyses on three different levels of pool control sera. The number, mean values, standard deviation and coefficient of variation for each of these control sera are presented in Table 2 and Table 3.

TABLE 2
Within Assay Precision (Values in pg/ml)

Sample	N	X	σ	C.V.
Low	20	81.9	8.1	9.9%
Normal	20	242.7	20.5	8.5%
High	20	423.7	7.5	7.5%

TABLE 3
Between Assay Precision (Values in pg/ml)

Sample	N	X	σ	C.V.
Low	20	106.1	5.1	4.8%
Normal	20	261.5	10.0	3.8%
High	20	436.7	13.5	8.2%

*As measured in ten experiments in duplicate over a ten day period.

14.2 Sensitivity

The estradiol AccuBind® EIA Test System has a sensitivity of 8.2 pg/ml. The sensitivity was ascertained by determining the variability of the 0 pg/ml serum calibrator and using the 2σ (95% certainty) statistic to calculate the minimum dose.

14.3 Accuracy

The Estradiol AccuBind® ELISA Test System was compared with a reference method. Biological specimens from low, normal and relatively high estradiol level populations were used (The values ranged from 10 pg/ml – 4300 pg/ml). The total number of such specimens was 65. The least square regression equation and the correlation coefficient were computed for this estradiol EIA in comparison with the reference method. The data obtained is displayed in Table 4.

TABLE 4

Method	Mean (x)	Least Square Regression Analysis	Correlation Coefficient
Monobind (y)	336.8	y= 36.50+1.023(x)	0.989
Reference (X)	293.4		

Only slight amounts of bias between this method and the reference method are indicated by the closeness of the mean values. The least square regression equation and correlation coefficient indicates excellent method agreement.

14.4 Specificity

The % cross reactivity of the estradiol antibody to selected substances was evaluated by adding the interfering substance to a serum matrix at various concentrations. The cross-reactivity was calculated by deriving a ratio between dose of interfering substance to dose of estradiol needed to displace the same amount of labeled analog.

Substance	Cross Reactivity
Androstenedione	0.0003
Dihydotestosterone	0.0008
Cortisone	<0.0001
Corticosterone	<0.0001
Cortisol	0.0004
Estriol	<0.0001
DHEA sulfate	<0.0001
Estradiol	<0.0001
Estrone	<0.0001
Testosterone	<0.0001

15.0 REFERENCES

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Revision: 5 Date: 2019-Jul-16 DCO: 1353
MP4925 Product Code: 4925-*300*

Size		96(A)	192(B)
Reagent (fill)	A)	1ml set	1ml set
	B)	1 (6ml)	2 (6ml)
	C)	1 (6ml)	2 (6ml)
	D)	1 plate	2 plates
	E)	1 (20ml)	1 (20ml)
	F)	1 (12ml)	2 (12ml)
	G)	1 (8ml)	2 (8ml)

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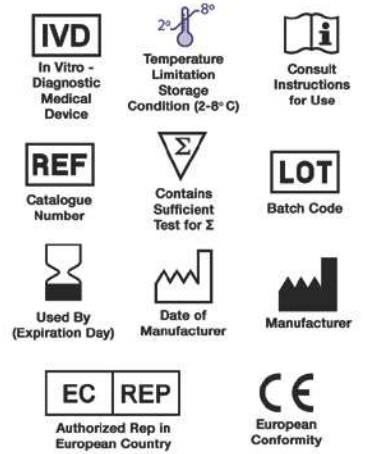
Glossary of Symbols
(EN 980/ISO 15223)



Monobind Inc.
Lake Forest, CA 92630, USA

AccuBind ELISA Microwells

Ferritin Test System
Product Code: 2825-300

1.0 INTRODUCTION

Intended Use: The Quantitative Determination of Circulating Ferritin Concentrations in Human Serum by a Microplate Enzyme Immunoassay, Colorimetric

2.0 SUMMARY AND EXPLANATION OF THE TEST

Ferritin, in circulation, as measured in serum levels is a satisfactory index of body's iron storage. The iron storage is directly measured by quantitative phlebotomy, iron absorption studies, liver biopsies and microscopic examinations of bone marrow aspirates. Iron deficiency (Anemia) and iron overload (Hemochromatosis) are conditions associated with body's iron storage or lack thereof. Measurements of total iron binding capacity (TIBC) have widely been used as aids in the determination of these conditions. However, an assay of serum Ferritin is simply more sensitive and reliable means of demonstration these disorders.

Ferritin is present in blood in very low concentrations. Normally, approximately 1% of plasma iron is contained in Ferritin. The plasma ferritin, is in equilibrium with body stores, and variations of iron storage. The plasma concentrations of ferritin decline very early in anemic conditions like development of iron deficiency, long before the changes are observed in the blood hemoglobin concentration, size of the erythrocytes and TIBC. Thus measurements of serum ferritin can serve as an early indicator of iron deficiency that is uncomplicated by other concurrent conditions. At the same time a large number of chronic conditions can result in elevated levels of serum ferritin. These include chronic infections, chronic inflammatory diseases such as rheumatoid arthritis, heart disease and some other malignancies, especially lymphomas, leukemia, breast cancer and neuroblastoma. In patients who have these chronic disorders together with iron deficiency, serum ferritin levels are often normal. An increase in circulating ferritin is observed in patients with viral hepatitis or after a toxic liver injury as a release of ferritin from the injured liver cells. Elevated serum ferritin levels are found in patients with hemochromatosis and hemosiderosis.

Circulating ferritin levels have been used by clinicians, as an aid, in the diagnosis of several other disorders. It has proved as a valuable tool in differential diagnosis of anemia due to iron deficiency and anemias due to other disorders and, in exposing the depletion of iron reserves long before the onset of anemia. Serial determinations have been used to monitor, non-invasively, the erosion of iron storage during pregnancy and in patients undergoing dialysis. Serum ferritin is routinely used as a screen for iron deficiency for a variety of populations like blood donors and people who are receiving regular blood transfusions or iron replacement therapy.

In this method, ferritin calibrator, patient specimen or control is first added to a streptavidin coated well. Biotinylated monoclonal antibody (specific for ferritin) is added and the reactants mixed. Reaction results between the biotinylated ferritin antibody and native ferritin to form an immune complex that is deposited on the streptavidin coated well. The excess serum proteins are washed away via a wash step. Another ferritin specific antibody, labeled with an enzyme, is added to the wells. The enzyme labeled antibody binds to the ferritin already immobilized on the well. Excess enzyme is washed off via a wash step. A color is generated by the addition of a substrate. The intensity of the color generation is directly proportional to the concentration of the ferritin in the sample.

The employment of several serum references of known ferritin levels permits the construction of a dose response curve of activity and concentration. From comparison to the dose response curve, an unknown specimen's activity can be correlated with ferritin concentration.

3.0 PRINCIPLE

Immunoenzymometric sequential assay (TYPE 4):
The essential reagents required for an immunoenzymometric assay include high affinity and specificity antibodies (enzyme and immobilized), with different and distinct epitope recognition, in excess, and native antigen. In this procedure, the immobilization takes place during the assay at the surface of a microplate well through the interaction of streptavidin coated on the well and exogenously added biotinylated monoclonal anti- *ferritin* antibody.

Upon mixing monoclonal biotinylated antibody, and a serum containing the native antigen, reaction results between the native antigen and the antibody, forming an antibody-antigen complex. Simultaneously the biotin attached to the antibody binds to the streptavidin coated on the microwells resulting in immobilization of the complex. The interaction is illustrated by the following equation:

$$Ag_{(ferritin)} + {}^{Btn}Ab_{(m)} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} Ag_{(ferritin)} - {}^{Btn}Ab_{(m)}$$

${}^{Btn}Ab_{(m)}$ = Biotinylated Monoclonal Antibody (Excess Quantity)
$Ag_{I(ferritin)}$ = Native Antigen (Variable Quantity)
$Ag_{(ferritin)} - {}^{Btn}Ab_{(m)}$= Antigen-Antibody complex (Variable Quan.))
k_a = Rate Constant of Association
k_{-a} = Rate Constant of Disassociation

$Ag_{(ferritin)} - {}^{Btn}Ab_{(m)}$ + $\underline{Streptavidin}_{C.W.} \Rightarrow \underline{immobilized\ complex}$ (IC)
$\underline{Streptavidin}_{C.W.}$ = Streptavidin immobilized on well
$\underline{Immobilized\ complex\ (IC)}$ = Ag-Ab bound to the well

After a suitable incubation period, the antibody-antigen bound fraction is separated from unbound antigen by decantation or aspiration. Another antibody (directed at a different epitope) labeled with an enzyme is added. Another interaction occurs to form an enzyme labeled antibody-antigen-biotinylated-antibody complex on the surface of the wells. Excess enzyme is washed off via a wash step. A suitable substrate is added to produce color measurable with the use of a microplate spectrophotometer. The enzyme activity on the well is directly proportional to the native antigen concentration. By utilizing several different serum references of known antigen concentration, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

$$(IC) + {}^{Enz}Ab_{(ferritin)} \underset{k_{-b}}{\overset{k_b}{\rightleftharpoons}} {}^{Enz}Ab_{(ferritin)} - IC$$

${}^{Enz}Ab_{(ferritin)}$ = Enzyme labeled Antibody (Excess Quantity)
${}^{Enz}Ab_{(ferritin)}$ – IC = Antigen-Antibodies Complex
k_b = Rate Constant of Association
k_{-b} = Rate Constant of Dissociation

4.0 REAGENTS

Materials Provided:

A. Ferritin Calibrators – 1ml / vial - Icons A-F
Six (6) vials of Ferritin calibrators at levels of 0(**A**), 10(**B**), 50(**C**), 150(**D**), 400(**E**) and 800(**F**) ng/ml. Store at 2-8°C. A preservative has been added.
Note: The calibrators, human serum based, were calibrated using a reference preparation, which was assayed against the WHO 3rd IS 94/572

B. Ferritin Biotin Reagent – 13ml/vial - Icon ∇
One (1) vial containing biotinylated monoclonal mouse IgG in buffer, dye, and preservative. Store at 2-8°C.

C. Ferritin Enzyme Reagent – 13 ml/vial-Icon
One (1) vial containing Horseradish Peroxidase (HRP) labeled anti-ferritin IgG in buffer, dye and preservatives. Store at 2-8°C.

D. Streptavidin Coated Plate – 96 wells – Icon ⇓
One 96-well microplate coated with streptavidin and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

E. Wash Solution Concentrate – 20 ml/vial - Icon
One (1) vial containing a surfactant in buffered saline. A preservative has been added. Store at 2-8°C.

F. Substrate A – 7ml/vial - Icon S^A
One (1) vial containing tetramethylbenzidine (TMB) in buffer. Store at 2-8°C.

G. Substrate B – 7ml/vial - Icon S^B
One (1) vial containing hydrogen peroxide (H_2O_2) in buffer. Store at 2-8°C.

H. Stop Solution – 8m/vial - Icon STOP
One (1) vial containing a strong acid (1N HCl). Store at 2-8°C.

I. Product Instructions.

Note 1: Do not use reagents beyond the kit expiration date.
Note 2: Avoid extended exposure to heat and light. **Opened reagents are stable for sixty (60) days when stored at 2-8°C. Kit and component stability are identified on the label.**
Note 3: Above reagents are for a single 96-well microplate. For other kit configurations, please refer to the table at the end of the instructions.

4.1 Required But Not Provided:

1. Pipette capable of delivering 0.025 and 0.050ml (25 & 50µl) volumes with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100 and 0.350ml (100 and 350µl) volumes with a precision of better than 1.5%.
3. Microplate washers or a squeeze bottle (optional).
4. Microplate Reader with 450nm and 620nm wavelength absorbance capability.
5. Absorbent Paper for blotting the microplate wells.
6. Plastic wrap or microplate cover for incubation steps.
7. Vacuum aspirator (optional) for wash steps.
8. Timer.
9. Quality control materials.

5.0 PRECAUTIONS

For In Vitro Diagnostic Use
Not for Internal or External Use in Humans or Animals
All products that contain human serum have been found to be non-reactive for Hepatitis B Surface Antigen, HIV 1&2 and HCV Antibodies by FDA licensed reagents. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Edition, 1988, HHS Publication No. (CDC) 88-8395.
Safe Disposal of kit components must be according to local regulatory and statutory requirement.

6.0 SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood serum in type and the usual precautions in the collection of venipuncture samples should be observed. For accurate comparison to established normal values, a fasting morning serum sample should be obtained. The blood should be collected in a plain redtop venipuncture tube without additives or anti-coagulants. Allow the blood to clot for samples. Centrifuge the specimen to separate the serum from the cells.

In patients receiving therapy with high biotin doses (i.e. >5mg/day), no sample should be taken until at least 8 hours after the last biotin administration, preferably overnight to ensure fasting sample.

Samples may be refrigerated at 2-8°C for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20°C for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.050ml (50µl) of the specimen is required.

7.0 QUALITY CONTROL

Each laboratory should assay controls at levels in the low, normal and elevated range for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

8.0 REAGENT PREPARATION

1. **Wash Buffer**
   Dilute contents of wash solution to 1000ml with distilled or deionized water in a suitable storage container. Store at 2-30°C for up to 60 days.
2. **Working Substrate Solution** – Stable for one year
   Pour the contents of the amber vial labeled Solution 'A' into the clear vial labeled Solution 'B'. Place the yellow cap on the clear vial for easy identification. Mix and label accordingly. Store at 2 - 8°C.

Note 1: Do not use the working substrate if it looks blue.
Note 2: Do not use reagents that are contaminated or have bacteria growth.

9.0 TEST PROCEDURE

Before proceeding with the assay, bring all reagents, serum reference calibrators and controls to room temperature (20-27°C).
*****Test Procedure should be performed by a skilled individual or trained professional*****

1. Format the microplates' wells for each serum reference, control and patient specimen to be assayed in duplicate. **Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.**
2. Pipette 0.025 ml (25µl) of the appropriate serum reference, control or specimen into the assigned well.
3. Add 0.100 ml (100µl) of the Ferritin Biotin Reagent to each well. **It is very important to dispense all reagents close to the bottom of the coated well.**
4. Swirl the microplate gently for 20-30 seconds to mix and cover.
5. Incubate 30 minutes at room temperature.
6. Discard the contents of the microplate by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper.
7. Add 350µl of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.**
8. Add 0.100 ml (100µl) of the Ferritin Enzyme Conjugate to each well.
   DO NOT SHAKE THE PLATE AFTER ENZYME ADDITION
9. Incubate 30 minutes at room temperature.
10. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.
11. Add 350µl of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes.
12. Add 0.100 ml (100µl) of working substrate solution to all wells (see Reagent Preparation Section).
    DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION
13. Incubate at room temperature for fifteen (15) minutes.
14. Add 0.050ml (50µl) of stop solution to each well and mix gently for 15-20 seconds.
15. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) in a microplate reader. **The results should be read within thirty (30) minutes of adding the stop solution.**

Note: Always add reagents in the same order to minimize reaction time differences between wells.

10.0 CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of *ferritin* in unknown specimens.

1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the absorbance for each duplicate serum reference versus the corresponding ferritin concentration in ng/ml on linear graph paper.
3. Draw the best-fit curve through the plotted points.
4. To determine the concentration of ferritin for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in ng/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance (1.287) intersects the dose response curve at 154 ng/ml ferritin concentration (See Figure 1).

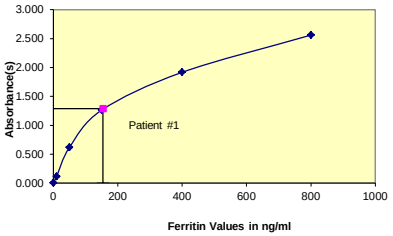
Note: Computer data reduction software designed for ELISA assays may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained.

EXAMPLE 1

Sample ID	Well	Abs	Mean Abs (B)	Conc
Cal A	A1	0.002	0.003	0
	B1	0.003		
Cal B	C1	0.110	0.112	10
	D1	0.113		
Cal C	E1	0.586	0.617	50
	F1	0.647		
Cal D	G1	1.204	1.262	150
	H1	1.320		
Cal E	A2	1.947	1.917	400
	B2	1.887		
Cal F	C2	2.586	2.561	800
	D2	2.536		
Ctrl 1	E2	0.707	0.721	***66.1***
	F2	0.734		
Patient 1	G2	1.289	1.287	***154.0***
	H2	1.285		
Patient 2	A3	1.647	1.659	***301.6***
	B3	1.671		

*The data presented in Example 1 and Figure 1 is for illustration only and **should not** be used in lieu of a dose response curve prepared with each assay.

Figure 1



11.0 Q. C. PARAMETERS

In order for the assay results to be considered valid the following criteria should be met.

1. The absorbance (OD) of Calibrator F should be ≥ 1.3
2. The absorbance of the A calibrator should be ≤ 0.05
3. Four out of six quality control pools should be within the established ranges.

12.0 RISK ANALYSIS

The MSDS and Risk Analysis Form for this product is available on request from Monobind Inc.

12.1 Assay Performance

1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. The addition of substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the substrate and stop solution should be added in the same sequence to eliminate any time-deviation during reaction.
6. Plate readers measure vertically. Do not touch the bottom of the wells.
7. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
8. Use components from the same lot. No intermixing of reagents from different batches.
9. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from Monobind IFU may yield inaccurate results.
10. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.
11. It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.
12. Risk Analysis- as required by CE Mark IVD Directive 98/79/EC - for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com.

12.2 Interpretation

1. ***Measurements and interpretation of results must be performed by a skilled individual or trained professional.***
2. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.
3. The reagents for the test system have been formulated to eliminate maximal interference; however, potential interaction between rare serum specimens and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for all kinds of immunoassays (Boscato LM, Stuart MC. 'Heterophilic antibodies: a problem for all immunoassays' Clin. Chem. 1988:3427-33). For diagnostic purposes, the results from this assay should be in combination with clinical examination, patient history and all other clinical findings.
4. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
5. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, Monobind shall have no liability.
6. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.
7. Patient specimens with *ferritin* concentrations above 800 ng/ml may be diluted (for example 1/10) with normal serum stripped of *ferritin* and re-assayed. The sample's concentration is obtained by multiplying the result by the dilution factor (10).
8. Each component in one assay should be of the same lot number and stored under identical conditions.

13.0 EXPECTED RANGE OF VALUES

Approximate reference ranges for normal males and female adults were established by using 400 normal sera with the Ferritin AccuBind® ELISA test system

Males	16-220 ng/ml
Females	10-124 ng/ml

In addition to the above the following ranges were assigned based on the available literature. However, these ranges were confirmed using AccuBind® Ferritin Microplate Elisa Procedure with limited number of samples.

Newborn	22-220 ng/ml
1-2 Months	190-610 ng/ml
2-5 Months	50-220 ng/ml
6Mos – 16 Yrs	10 – 160 ng/ml

It is important to keep in mind that any normal range establishment is dependent upon a multiplicity of factors like the specificity of the method, the locale, the population tested and the precision of the method in the hands of technicians. For these reasons each laboratory should depend upon the range of expected values established by the manufacturer only until an in-house range can be determined by the technicians using the method with a population indigenous to the area in which the laboratory is located.

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

The within and between assay precisions of the ferritin AccuBind® ELISA test system were determined by analyses on three different levels of control sera. The number (N), mean value (X), standard deviation (σ) and coefficient of variation (C.V.) for each of these control sera are presented in Table 2 and Table 3.

TABLE 2
Within Assay Precision (Values in ng/ml)

Sample	N	X	σ	C.V.
Level 1	20	43.5	1.36	3.1%
Level 2	20	110.5	6.10	5.5%
Level 3	20	349.6	7.54	2.2%

TABLE 3
Between Assay Precision* (Values in ng/ml)

Sample	N	X	σ	C.V.
Level 1	10	41.2	2.33	5.5%
Level 2	10	113.2	8.11	7.2%
Level 3	10	372.4	11.80	3.2%

*As measured in ten experiments in duplicate.

14.2 Sensitivity

The minimum detectable dose (*Sensitivity*) is defined as the apparent concentration 2 σ above the absorbance for zero calibrator. 2 σ of the mean absorbance for twenty replicates for zero calibrator for the ferritin AccuBind™ ELISA test system gave a sensitivity of 0.17 ng/ml.

14.3 Specificity

The cross-reactivity of the ferritin AccuBind® ELISA test system to selected substances was evaluated by adding the interfering substance to a serum matrix at various concentrations. The cross-reactivity was calculated by deriving a ratio between dose of interfering substance to dose of Ferritin needed to produce the same absorbance.

Substance	Cross Reactivity
Liver Ferritin	100%
Spleen Ferritin	100%
Human Heart Ferritin	<1.0%
Hemoglobin	<0.1%

14.4 High Dose Effect

Since the assay is sequential in design, high concentrations of ferritin do not show the hook effect. Samples with concentrations over 50,000 ng/ml demonstrated extremely high levels of absorbance

15.0 REFERENCES

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Revision: 5 Date: 2022-Mar-30 DCO: 1543
MP2825 Product Code: *2825-300*

Size		96(A)	192(B)
Reagent (fill)	A)	1ml set	1ml set
	B)	1 (13ml)	2 (13ml)
	C)	1 (13ml)	2 (13ml)
	D)	1 plate	2 plates
	E)	1 (20ml)	1 (20ml)
	F)	1 (7ml)	2 (7ml)
	G)	1 (7ml)	2 (7ml)
	H)	1 (8ml)	2 (8ml)

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IVD CE
EC REP CEpartner4U, Esdoornlaan 13 3951 DB Maarn, The Nosthorlands www.cepartner4u.eu

Please visit our website to learn more about our products and services.

Glossary of Symbols
(EN 980/ISO 15223)

IVD In Vitro - Diagnostic Medical Device
Temperature Limitation Storage Condition (2-8° C)
Consult Instructions for Use
REF Catalogue Number
Contains Sufficient Test for Σ
LOT Batch Code
Used By (Expiration Day)
Date of Manufacturer
Manufacturer
EC REP Authorized Rep in European Country
CE European Conformity

HBe Ag&Ab

Enzyme Immunoassay (ELISA) for the determination of Hepatitis B Virus "e" Antigen and Antibody in human plasma and sera.

- for “in vitro” diagnostic use only -

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REF. HBE.CE
96 Tests

HBe Ag&Ab

A. INTENDED USE

Enzyme ImmunoAssay (ELISA) for the determination of Hepatitis B Virus "e" Antigen and Antibody in human plasma and sera.

The kit is intended for the follow-up of acute infection and of chronic patients under therapy.

For “in vitro” diagnostic use only.

B. INTRODUCTION

Hepatitis B “e” Antigen or HBeAg is known to be intimately associated with Hepatitis B Virus or HBV replication and the presence of infectious Dane particles in the blood.

Recently, it has been found that HBeAg is a product of proteolytic degradation of Hepatitis B core Antigen or HBcAg, occurring in hepatocites, whose expression is under the control of the precore region of HBV genome.

If HBeAg is considered a specific marker of infectivity, the presence of anti HBeAg antibodies in blood is recognised to be a clinical sign of recovery from infection to convalescence.

The determination of these two analytes in samples from HBV patients has become important for the classification of the phase of illness and as a prognostic value in the follow up of infected patients.

C. PRINCIPLE OF THE TEST

HBeAg:

HBeAg, if present in the sample, is captured by a specific monoclonal antibody, in the 1st incubation.

In the 2nd incubation, after washing, a tracer, composed of a mix of two specific anti HBeAg monoclonal antibodies, labeled with peroxidase (HRP), is added to the microplate and binds to the captured HBeAg.

The concentration of the bound enzyme on the solid phase is proportional to the amount of HBeAg in the sample and its activity is detected by adding the chromogen/substrate in the 3rd incubation.

The presence of HBeAg in the sample is determined by means of a cut-off value that allows for the semiquantitative detection of the antigen.

HBeAb

Anti HBeAg antibodies, if present in the sample, compete with a recombinant HBeAg preparation for a fixed amount of an anti HBeAg antibody, coated on the microplate wells.

The competitive assay is carried out in two incubations, the first with the sample and recHBeAg, and the second with a tracer, composed of two anti HBeAg monoclonal antibodies, labeled with peroxidase (HRP).

The concentration of the bound enzyme on the solid phase becomes inversely proportional to the amount of anti HBeAg antibodies in the sample and its activity is detected by adding the chromogen/substrate in the third incubation.

The concentration of HBeAg specific antibodies in the sample is determined by means of a cut-off value that allows for the semi quantitative detection of anti HBeAg antibodies.

D. COMPONENTS

The kit contains reagents for total 96 tests.

1. Microplate: MICROPLATE

n° 1 coated microplate

12 strips of 8 breakable wells coated with anti HBeAg specific monoclonal antibody, postcoated with bovine serum proteins and sealed into a bag with desiccant. Allow the microplate to reach room temperature before opening; reseal unused strips in the bag with desiccant and store at 2..8°C.

2. Negative Control: CONTROL -

1x2.0ml/vial. Ready to use control. It contains bovine serum, 0.09% sodium azide and 0.045% ProClin 300 as preservatives. The negative control is colorless.

3. Antigen Positive Control: CONTROL + Ag

1x1.0ml/vial. Ready to use control. It contains 2% bovine serum albumin, non infectious recombinant HBeAg, 100 mM tris buffer pH 7.4+/-0.1, 0.09% sodium azide and 0.045% ProClin 300 as preservatives.

The positive control is green color coded.

4. Antibody Positive Control: **:** CONTROL + Ab

1x1.0ml/vial. Ready to use control. It contains 2% bovine serum albumin, human anti HbeAg positive plasma at about 10 PEI U/ml, 100 mM tris buffer pH 7.4+/-0.1, 0.09% sodium azide and 0.045% ProClin 300 as preservatives. The label is red colored.

The positive control is yellow color coded.

5. Antigen Calibrator: CALAG …ml

n° 1 vial. Lyophilised calibrator for HBeAg. To be dissolved with EIA grade water as reported in the label. It contains fetal bovine serum, non infectious recombinant HBeAg at 1 PEI U/ml +/- 10%, 0.02% gentamicine sulphate and 0.045% ProClin 300 as preservatives.

Important Note: The volume necessary to dissolve the content of the vial may vary from lot to lot. Please use the right volume reported on the label.

6. Antibody Calibrator: CALAB …ml

n° 1 vial. Lyophilized calibrator for anti HBeAg antibody. To be dissolved with EIA grade water as reported in the label. It contains fetal bovine serum, positive plasma at 0.25 PEI U/ml +/-10%, 0.02% gentamicine sulphate and 0.045% ProClin 300 as preservatives. The label is red colored.

Important Note: The volume necessary to dissolve the content of the vial may vary from lot to lot. Please use the right volume reported on the label.

7. Wash buffer concentrate: WASHBUF 20X

1x60ml/bottle. 20x concentrated solution.

Once diluted, the wash solution contains 10 mM phosphate buffer pH 7.0+/-0.2, 0.05% Tween 20 and 0.045% ProClin 300.

8. Enzyme conjugate: CONJ

1x16ml/vial. Ready to use conjugate. It contains Horseradish peroxidase conjugated with a mix of monoclonal antibodies to HBeAg, 10 mM Tris buffer pH 6.8+/-0.1, 2% BSA, 0.045% ProClin 300 and 0.02% gentamicine sulphate as preservatives.

The reagent is red color coded.

9. HBe Antigen: Ag-HBe

1x10ml/vial. Ready to use reagent. It contains recombinant HBeAg, fetal bovine serum, buffered solution pH 8.0+/-0.1, 0.045% ProClin 300 and 0.09% sodium azide as preservatives.

The reagent is blue color coded.

10. Chromogen/Substrate: SUBS TMB

1x16ml/vial. Ready-to-use component. It contains a 50 mM citrate-phosphate buffered solution at pH 3.5-3.8, 4% dimethylsulphoxide, 0.03% tetra-methyl-benzidine or TMB and 0.02% hydrogen peroxide or H_2O_2.

Note: To be stored protected from light as sensitive to strong illumination.

11. Sulphuric Acid: H_2SO_4 O.3 M

1x15ml/vial. It contains 0.3 M H2SO4 solution.

Attention: Irritant (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

12. Plate sealing foils n°2

13. Package insert n°1

E. MATERIALS REQUIRED BUT NOT PROVIDED

1. Calibrated Micropipettes (150ul, 100ul and 50ul) and disposable plastic tips.
2. EIA grade water (double distilled or deionised, charcoal treated to remove oxidizing chemicals used as disinfectants).
3. Timer with 60 minute range or higher.
4. Absorbent paper tissues.
5. Calibrated ELISA microplate thermostatic incubator (dry or wet) set at +37°C.
6. Calibrated ELISA microwell reader with 450nm (reading) and with 620-630nm (blanking) filters.
7. Calibrated ELISA microplate washer.
8. Vortex or similar mixing tools.

F. WARNINGS AND PRECAUTIONS

1. The kit has to be used by skilled and properly trained technical personnel only, under the supervision of a medical doctor responsible of the laboratory.
2. All the personnel involved in performing the assay have to wear protective laboratory clothes, talc-free gloves and glasses. The use of any sharp (needles) or cutting (blades) devices should be avoided. All the personnel involved should be trained in biosafety procedures, as recommended by the Center for Disease Control, Atlanta, U.S. and reported in the National Institute of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
3. All the personnel involved in sample handling should be vaccinated for HBV and HAV, for which vaccines are available, safe and effective.
4. The laboratory environment should be controlled so as to avoid contaminants such as dust or air-born microbial agents, when opening kit vials and microplates and when performing the test. Protect the Chromogen/Substrate (TMB) from strong light and avoid vibration of the bench surface where the test is undertaken.
5. Upon receipt, store the kit at 2-8°C into a temperature controlled refrigerator or cold room.
6. Do not interchange components between different lots of the kits. It is recommended that components between two kits of the same lot should not be interchanged.
7. Check that the reagents are clear and do not contain visible heavy particles or aggregates. If not, advise the laboratory supervisor to initiate the necessary procedures.
8. Avoid cross-contamination between serum/plasma samples by using disposable tips and changing them after each sample. Do not reuse disposable tips.
9. Avoid cross-contamination between kit reagents by using disposable tips and changing them between the use of each one. Do not reuse disposable tips.
10. Do not use the kit after the expiration date stated on external (primary container) and internal (vials) labels.
11. Treat all specimens as potentially infective. All human serum specimens should be handled at Biosafety Level 2, as recommended by the Center for Disease Control, Atlanta, U.S. in compliance with what reported in the Institutes of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
12. The use of disposable plastic-ware is recommended in the preparation of the washing solution or in transferring components into other containers of automated workstations, in order to avoid contamination.
13. Waste produced during the use of the kit has to be discarded in compliance with national directives and laws concerning laboratory waste of chemical and biological substances. In particular, liquid waste generated from the washing procedure, from residuals of controls and from samples has to be treated as potentially infective material and inactivated. Suggested procedures of inactivation are treatment with a 10% final concentration of household bleach for 16-18 hrs or heat inactivation by autoclave at 121°C for 20 min..
14. Accidental spills have to be adsorbed with paper tissues soaked with household bleach and then with water. Tissues should then be discarded in proper containers designated for laboratory/hospital waste.
15. The Stop Solution is an irritant. In case of spills, wash the surface with plenty of water
16. Other waste materials generated from the use of the kit (example: tips used for samples and controls, used microplates) should be handled as potentially infective and disposed according to national directives and laws concerning laboratory wastes.

G. SPECIMEN: PREPARATION AND RECOMMANDATIONS

1. Blood is drawn aseptically by venepuncture and plasma or serum is prepared using standard techniques of preparation of samples for clinical laboratory analysis. No influence has been observed in the preparation of the sample with citrate, EDTA and heparin.
2. Avoid any addition of preservatives; especially sodium azide as this chemical would affect the enzymatic activity of the conjugate, generating false negative results.
3. Samples have to be clearly identified with codes or names in order to avoid misinterpretation of results.
4. Haemolysed and visibly hyperlipemic ("milky") samples have to be discarded as they could generate false results. Samples containing residues of fibrin or heavy particles or microbial filaments and bodies should be discarded as they could give rise to false results.
5. Sera and plasma can be stored at +2°…+8°C in primary collection tubes for up to five days after collection.
Do not freeze primary tubes of collection. For longer storage periods, sera and plasma samples, carefully removed from the primary collection tube, can be stored frozen at –20°C for at least 12 months. Any frozen samples should not be frozen/thawed more than once as this may generate particles that could affect the test result.
6. If particles are present, centrifuge at 2.000 rpm for 20 min or filter using 0.2-0.8u filters to clean up the sample for testing.

H. PREPARATION OF COMPONENTS AND WARNINGS

A study conducted on an opened kit has not pointed out any relevant loss of activity up to 6 re-uses of the device and up to 3 months.

1. Microplate:
Allow the microplate to reach room temperature (about 1 hr) before opening the container. Check that the desiccant has not turned dark green, indicating a defect in manufacturing.
In this case, call Dia.Pro's customer service.
Unused strips have to be placed back into the aluminum pouch, with the desiccant supplied, firmly zipped and stored at +2°-8°C.
When opened the first time, unused strips are stable until the humidity indicator inside the desiccant bag turns from yellow to green.

2. Negative Control:
Ready to use. Mix well on vortex before use.

3. Antigen Positive Control:
Ready to use. Mix well on vortex before use.

4. Antibody Positive Control:
Ready to use. Mix well on vortex before use.

5. Antigen Calibrator:
Add the volume of ELISA grade water, reported on the label, to the lyophilized powder; let fully dissolve and then gently mix on vortex.

Note: The dissolved calibrator is not stable. Store it frozen in aliquots at –20°C.

6. Antibody Calibrator:
Add the volume of ELISA grade water, reported on the label, to the lyophilized powder; let fully dissolve and then gently mix on vortex.
Note: The dissolved calibrator is not stable. Store it frozen in aliquots at –20°C.

7. Wash buffer concentrate:
The whole content of the 20x concentrated solution has to be diluted with bidistilled water up to 1200 ml and mixed gently end-over-end before use.
During preparation avoid foaming as the presence of bubbles could impact on the efficiency of the washing cycles.
Note: Once diluted, the wash solution is stable for 1 week at +2..8° C.

8. Enzyme conjugate:
Ready to use. Mix well on vortex before use.
Avoid contamination of the liquid with oxidizing chemicals, air-driven dust or microbes. If this component has to be transferred, use only plastic, and if possible, sterile disposable containers.

9. HBe Antigen:
Ready to use. Mix well on vortex before use.
Avoid contamination of the liquid with oxidizing chemicals, air-driven dust or microbes. If this component has to be transferred, use only plastic, and if possible, sterile disposable containers.

10. Chromogen/Substrate:
Ready to use. Mix well on vortex before use.
Avoid contamination of the liquid with oxidizing chemicals, air-driven dust or microbes. Do not expose to strong light, oxidizing agents and metallic surfaces.
If this component has to be transferred use only plastic, and if possible, sterile disposable container.

11. Sulphuric Acid:
Ready to use. Mix well on vortex before use.
Attention: Irritant (H315, H319; P280, P302+P352, P332+P313, P305+P351+P338, P337+P313, P362+P363).

Legenda:

Warning **H statements:**
H315 – Causes skin irritation.
H319 – Causes serious eye irritation.

Precautionary **P statements:**
P280 – Wear protective gloves/protective clothing/eye protection/face protection.

P302 + P352 – IF ON SKIN: Wash with plenty of soap and water.
P332 + P313 – If skin irritation occurs: Get medical advice/attention.
P305 + P351 + P338 – IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P337 + P313 – If eye irritation persists: Get medical advice/attention.
P362 + P363 – Take off contaminated clothing and wash it before reuse.

I. INSTRUMENTS AND TOOLS USED IN COMBINATION WITH THE KIT

1. Micropipettes have to be calibrated to deliver the correct volume required by the assay and must be submitted to regular decontamination (household alcohol, 10% solution of bleach, hospital grade disinfectants) of those parts that could accidentally come in contact with the sample. Decontamination of spills or residues of kit components should also be carried out regularly. They should also be regularly maintained in order to show a precision of 1% and a trueness of $\pm$2%.
2. The ELISA incubator has to be set at +37°C (tolerance of +/- 0.5°C) and regularly checked to ensure the correct temperature is maintained. Both dry incubators and water baths are suitable for the incubations, provided that the instrument is validated for the incubation of ELISA tests.
3. The **ELISA washer** is extremely important to the overall performances of the assay. The washer must be carefully validated in advance, checked for the delivery of the right dispensation volume and regularly submitted to maintenance according to the manufacturer's instructions for use. In particular the washer, at the end of the daily workload, has to be extensively cleaned out of salts with deionized water. Before use, the washer has to be extensively primed with the diluted Washing Solution.
   The instrument weekly has to be submitted to decontamination according to its manual (NaOH 0.1 M decontamination suggested).
   5 washing cycles (aspiration + dispensation of 350ul/well of washing solution + 20 sec soaking = 1 cycle) are sufficient to ensure the assay with the declared performances. If soaking is not possible add one more cycle of washing.
   An incorrect washing cycle or salt-blocked needles are the major cause of false positive reactions.
4. Incubation times have a tolerance of $\pm$5%.
5. The ELISA reader has to be equipped with a reading filter of 450nm and with a second filter of 620-630nm, mandatory for blanking purposes. Blanking is carried out on the well identified in the section "Assay Procedure". The optical system of the reader has to be calibrated regularly to ensure the correct optical density is measured. It should be regularly maintained according to the manufacturer 's instructions.
6. When using an ELISA automated work station, all critical steps (dispensation, incubation, washing, reading, data handling) have to be carefully set, calibrated, controlled and regularly serviced in order to match the values reported in the section "Internal Quality Control''. The assay protocol has to be installed in the operating system of the unit and validated as for the washer and the reader. In addition, the liquid handling part of the station (dispensation and washing) has to be validated and correctly set. Particular attention must be paid to avoid carry over by the needles used for dispensing and for washing. This must be studied and controlled to minimize the possibility of contamination of adjacent wells. The use of ELISA automated work stations is recommended when the number of samples to be tested exceed 20-30 units per run.
7. Dia.Pro's customer service offers support to the user in the setting and checking of instruments used in combination with the kit, in order to assure compliance with the requirements described. Support is also provided for the installation of new instruments to be used with the kit.

L. PRE ASSAY CONTROLS AND OPERATIONS

1. Check the expiration date of the kit printed on the external label (primary container). Do not use if expired.
2. Check that the liquid components are not contaminated by visible particles or aggregates. Check that the Chromogen/Substrate (TMB+H_2O_2) is colourless or pale blue by aspirating a small volume of it with a sterile plastic pipette. Check that no breakage occurred in transportation

and no spillage of liquid is present inside the box (primary container). Check that the aluminium pouch, containing the microplate, is not punctured or damaged.
3. Dilute all the content of the 20x concentrated Wash Solution as described above.
4. Dissolve the Calibrator as described above and gently mix.
5. Allow all the other components to reach room temperature (about 1 hr) and then mix gently on vortex all liquid reagents.
6. Set the ELISA incubator at +37°C and prepare the ELISA washer by priming with the diluted washing solution, according to the manufacturers instructions. Set the right number of washing cycles as reported in the specific section.
7. Check that the ELISA reader is turned on or ensure it will be turned on at least 20 minutes before reading.
8. If using an automated work station, turn on, check settings and be sure to use the right assay protocol.
9. Check that the micropipettes are set to the required volume.
10. Check that all the other equipment is available and ready to use.

In case of problems, do not proceed further with the test and advise the supervisor.

M. ASSAY PROCEDURE

The assay has to be performed according to the procedure given below, taking care to maintain the same incubation time for all the samples being tested.

A) HBe Antigen:

1. Place the required number of strips in the plastic holder and carefully identify the wells for controls, calibrator and samples.
2. Leave the A1 well empty for blanking purposes.
3. Pipette 100 µl of the Negative Control in triplicate, 100 µl of the Antigen Calibrator in duplicate and then 100 µl of the Antigen Positive Control in single.
4. Then dispense 100 µl of samples in the proper wells.
5. Check for the presence of samples in wells by naked eye (there is a marked colour difference between empty and full wells) or by reading at 450/620nm (samples show OD values higher than 0.100).
6. Incubate the microplate for **60 min at +37°C**.

Important note: *Strips have to be sealed with the adhesive sealing foil, only when the test is performed manually. Do not cover strips when using ELISA automatic instruments.*

7. When the first incubation is finished, wash the microwells as previously described (section I.3)
8. Dispense 100 µl Enzyme Conjugate in all wells, except for A1, used for blanking operations.

Important note: *Be careful not to touch the inner surface of the well with the pipette tip and not to immerse the top of it into samples or controls. Contamination might occur.*

9. Check that the reagent has been dispensed properly and then incubate the microplate for **60 min at +37°C**.
10. When the second incubation is finished, wash the microwells as previously described (section I.3)
11. Pipette 100 µl Chromogen/Substrate into all the wells, A1 included.

Important note: *Do not expose to strong direct light as a high background might be generated.*

12. Incubate the microplate protected from light at **room temperature (18-24°C) for 20 minutes**. Wells dispensed with positive control and positive samples will turn from clear to blue.
13. Pipette 100 µl Sulphuric Acid into all the wells using the same pipetting sequence as in step 11. Addition of the stop solution will turn the positive control and positive samples from blue to yellow.
14. Measure the color intensity of the solution in each well, as described in section I.5 using a 450nm filter (reading) and a 620-630nm filter (background subtraction, mandatory), blanking the instrument on A1.

B) HBe Antibody:

1. Place the required number of strips in the plastic holder and carefully identify the wells for controls, calibrator and samples.
2. Leave the A1 well empty for blanking purposes.
3. Pipette 50 µl of the Negative Control in triplicate, 50 µl of the Antibody Calibrator in duplicate and then 50 µl of the Antibody Positive Control in single.
4. Then dispense 50 µl of samples in the proper wells.
5. Check for the presence of samples in wells by naked eye (there is a marked color difference between empty and full wells) or by reading at 450/620nm (samples show OD values higher than 0.100).
6. Dispense then 50 µl of HBe Antigen in all the wells, except for A1.
7. Incubate the microplate for **60 min at +37°C**.

Important note: *Strips have to be sealed with the adhesive sealing foil, only when the test is performed manually. Do not cover strips when using ELISA automatic instruments.*

8. When the first incubation is finished, wash the microwells as previously described (section I.3)
9. Finally proceed as described for the HBeAg assay from point 8 to the last one.

Important notes:

1. *Ensure that no finger prints are present on the bottom of the microwell before reading. Finger prints could generate false positive results on reading.*
2. *Reading should ideally be performed immediately after the addition of the Stop Solution but definitely no longer than 20 minutes afterwards. Some self oxidation of the chromogen can occur leading to a higher background.*
3. *The Calibrator (CAL) does not affect the cut-off calculation and therefore the test results calculation. The Calibrator may be used only when a laboratory internal quality control is required by the management.*

N. ASSAY SCHEME

HBe antigen test

Controls and calibrator Samples	100 ul 100 ul
1st incubation	**60 min**
Temperature	+37°C
Wash step	n° 5 cycles with 20'' of soaking OR n° 6 cycles without soaking
Enzyme Conjugate	100 ul
2nd incubation	**60 min**
Temperature	+37°C
Wash step	n° 5 cycles with 20'' of soaking OR n° 6 cycles without soaking
TMB/H2O2 mix	100 ul
3rd incubation	**20 min**
Temperature	r.t.
Sulphuric Acid	100 ul
Reading OD	450nm/620-630nm

HBe antibody test

Controls and calibrator Samples	50 ul 50 ul
Neutralising antigen	50 ul
1st incubation	**60 min**
Temperature	+37°C
Wash step	n° 5 cycles with 20'' of soaking OR n° 6 cycles without soaking
Enzymatic conjugate	100 ul
2nd incubation	**60 min**
Temperature	+37°C
Wash step	n° 5 cycles with 20'' of soaking OR n° 6 cycles without soaking
TMB/H2O2 mixture	100 ul
3rd incubation	**20 min**
Temperature	r.t.
Sulphuric Acid	100 ul
Reading OD	450nm/620-630nm

An example of dispensation scheme is reported below:

Microplate

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	S2										
B	NC	S3										
C	NC	S4										
D	NC	S5										
E	CAL	S6										
F	CAL	S7										
G	PC	S8										
H	S1	S9										

Legenda: BLK = Blank // NC = Negative Control
PC = Positive Control // CAL = Calibrators // S = Sample

O. INTERNAL QUALITY CONTROL

A validation check is carried out on the controls any time the kit is used in order to verify whether the performances of the assay are as qualified.

Control that the following data are matched:

HBe Antigen

Check	OD450nm
Blank well	< 0.100 OD450nm
Negative Control (NC)	< 0.150 OD450nm after blanking coefficient of variation < 30%
Antigen Calibrator	S/Co > 2.0
Positive Control (PC)	> 1.500 OD450nm

HBe Antibody

Check	OD450nm
Blank well	< 0.100 OD450nm
Negative Control (NC)	> 1.000 OD450nm after blanking coefficient of variation < 10%
Antibody Calibrator	OD450nm < NC/1.5
Positive Control (PC)	OD450nm < NC/10

If the results of the test match the requirements stated above, proceed to the next section.

If they do not, don't proceed any further and perform the following checks:

HBeAg

Problem	Check
Blank well > 0.100 OD450nm	1. that the Chromogen/Substrate solution has not become contaminated during the assay
Negative Control (NC) > 0.150 OD450nm after blanking coefficient of variation > 30%	1. that the washing procedure and the washer settings are as validated in the pre qualification study; 2. that the proper washing solution has been used and the washer has been primed with it before use; 3. that no mistake has been done in the assay procedure (dispensation of positive control instead of negative control); 4. that no contamination of the negative control or of the wells where the control was dispensed has occurred due to positive samples, to spills or to the enzyme conjugate; 5. that micropipettes have not become contaminated with positive samples or with the enzyme conjugate 6. that the washer needles are not blocked or partially obstructed.
Calibrator S/Co < 2	1. that the procedure has been correctly performed; 2. that no mistake has occurred during its distribution (ex.: dispensation of negative control instead); 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the calibrator has occurred.
Positive Control < 1.500 OD450nm	1. that the procedure has been correctly performed; 2. that no mistake has occurred during the distribution of the control (dispensation of negative control instead of positive control); 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the positive control has occurred.

HBe antibody

Problem	Check
Blank well > 0.100 OD450nm	1. that the Chromogen/Substrate solution has not become contaminated during the assay
Negative Control (NC) < 1.000 OD450nm after blanking coefficient of variation > 10%	1. that the washing procedure and the washer settings are as validated in the pre qualification study; 2. that the proper washing solution has been used and the washer has been primed with it before use; 3. that no mistake has been done in the assay procedure (e.g.: dispensation of positive control instead of negative control; no dispensation of the Neutralizing Antigen; no dispensation of the Enzyme Conjugate); 4. that no contamination of the negative control or of the wells where the control was dispensed has occurred; 5. that micropipettes have not become contaminated with positive samples; 6. that the washer needles are not blocked or partially obstructed.

Calibrator OD450nm > NC/1.5	1. that the procedure has been correctly performed; 2. that no mistake has occurred during its distribution (ex.: dispensation of negative control instead; no dispensation of the Neutralizing Antigen; no dispensation of the Enzyme Conjugate); 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the calibrator has occurred.
Positive Control OD450nm > NC/10	1. that the procedure has been correctly performed; 2. that no mistake has occurred during the distribution of the control; 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination of the positive control has occurred.

If any of the above problems have occurred, report the problem to the supervisor for further actions.

Important note:
The analysis must be done proceeding as the reading step described in the section M, point 14.

P. CALCULATION OF THE CUT-OFF
The results are calculated by means of a cut-off value determined with the following formula:

HBeAg:

NC + 0.100 = Cut-Off (Co)

The value found for the test is used for the interpretation of results as described in the next paragraph.

HBeAb:

(NC + PC) / 3 = Cut-Off (Co)

***Important note**: When the calculation of results is performed by the operating system of an ELISA automated work station, ensure that the proper formulation is used to calculate the cut-off value and generate the correct interpretation of results.*

Q. INTERPRETATION OF RESULTS
Results are interpreted as follows:

HBeAg:

S/Co	Interpretation
< 0.9	Negative
0.9 - 1.1	Equivocal
> 1.1	Positive

HBeAb:

Co/S	Interpretation
< 0.9	Negative
0.9 - 1.1	Equivocal
> 1.1	Positive

Note:
S = OD450nm/620-630nm of the sample
Co = cut-off value

An example of calculation for HBeAg assay is reported below (data obtained proceeding as the the reading step described in the section M, point 14):

The following data must not be used instead or real figures obtained by the user.

Negative Control: 0.020 – 0.030 – 0.025 OD450nm
Mean Value: 0.025 OD450nm
Lower than 0.150 – Accepted

Positive Control: 2.489 OD450nm
Higher than 1.500 – Accepted
Cut-Off = 0.025+0.100 = 0.125
Calibrator: 0.520 - 0.540 OD450nm
Mean value: 0.530 OD450nm S/Co = 4.2
S/Co higher than 2.0 – Accepted

Sample 1: 0.030 OD450nm
Sample 2: 1.800 OD450nm
Sample 1 S/Co < 0.9 = negative
Sample 2 S/Co > 1.1 = positive

An example of calculation for HBeAb is reported below (data obtained proceeding as the the reading step described in the section M, point 14):

The following data must not be used instead or real figures obtained by the user.

Negative Control: 2.100 – 2.200 – 2.000 OD450nm
Mean Value: 2.100 OD450nm
Higher than 1.000 – Accepted

Positive Control: 0.100 OD450nm
Lower than NC/10 – Accepted

Cut-Off = (2.100 + 0.100) / 3 = 0.733
Calibrator: 0.720-0.760 OD450nm
Mean value: 0.740 OD450nm
OD450nm < NC/1.5 – Accepted

Sample 1: 0.020 OD450nm
Sample 2: 1.900 OD450nm
Sample 1 Co/S > 1.1 positive
Sample 2 Co/S < 0.9 negative

Important notes:
1. *Interpretation of results should be done under the supervision of the laboratory director to reduce the risk of judgment errors and misinterpretations.*
2. *The Identification of the clinical status of a HBV patient (acute, chronic, asymptomatic hepatitis) has to be done on the basis also of the other markers of HBV infection (HBsAg, HBsAb, HBcAb, HBcIgM);*
3. *When test results are transmitted from the laboratory to another facility, attention must be paid to avoid erroneous data transfer.*
4. *Diagnosis of viral hepatitis infection has to be taken by and released to the patient by a suitably qualified medical doctor.*

R. PERFORMANCE CHARACTERISTICS

A) HBeAg

1. Limit of detection
The limit of detection of the assay has been calculated by means of the International Standard for HBeAg, supplied by Paul Erlich Institute (PEI).
The data obtained by examining the limit of detection on three lots is reported in the table below.

HBE.CE Lot ID	PEI U/ml HBeAg
0103	0.25
0103/2	0.25
0303	0.25

In addition the preparation Accurun # 51, produced by Boston Biomedica Inc., USA, has been tested, upon dilution in FCS. Results are reported for three lots of products.

BBI's Accurun 51 (S/Co)

HBE.CE Lot ID	1 x	2 x	4 x	8 x	16x
0103	**4.1**	**1.6**	0.9	0.6	0.4
0103/2	**4.1**	**1.7**	0.9	0.6	0.4
0303	**4.0**	**1.6**	0.9	0.5	0.4

2. Diagnostic Sensitivity:
The diagnostic sensitivity has been tested on panels of samples classified positive by a US FDA approved kit.
Positive samples were collected from different HBV pathologies (acute, chronic) bearing HBeAg reactivity.
An overall value > 98% has been found in the study conducted on a total number of more than 200 samples.
Moreover the Panel of Seroconversion code PHM 935B, produced by BBI, was examined.
Data are reported below and compared with those reported by BBI for two other commercial products.

Sample ID	HBE.CE S/Co	Abbott EIA S/Co	Sorin EIA S/Co
21	5.4	4.5	6.3
22	3.7	4.3	5.4
23	1.9	3.2	3.1
24	1.1	2.4	1.5
25	1.0	2.1	1.2
26	0.6	1.7	0.7
27	0.2	0.8	0.3
28	0.2	0.6	0.2
29	0.2	0.4	0.2
30	0.2	0.3	0.2
31	0.1	0.3	0.2
32	0.1	0.3	0.2

Finally the Performance Panel code PHJ 201, produced by BBI, was tested. Data are reported below and compared with those reported by BBI for an other commercial product.

Member	PEI U/ml	HBE.CE	Sorin EIA
1	3	3.3	7.0
2	6	17.5	21.9
3	26	30.1	37.1
4	31	29.4	23.5
5	1	1.1	2.2
6	2	2.3	6.9
7	35	30.1	24.6
8	38	29.2	31.9
9	4	16.6	10.8
10	-	0.3	0.2
11	1	3.4	3.6
12	< 1	0.2	1.2
13	< 1	0.9	1.4
14	-	0.2	0.2
15	-	0.4	0.1
16	-	0.5	0.1
17	-	0.3	0.2
18	-	0.2	0.2
19	-	0.2	0.1
20	-	0.2	0.1
21	-	0.3	1.0
22	-	0.3	0.1
23	-	0.4	0.1
24	-	0.2	0.2
25	-	0.3	0.2

3. Diagnostic Specificity:
The diagnostic specificity has been determined on panels of negative samples from normal individuals and blood donors, classified negative with a FDA approved kit.
Both plasma, derived with different standard techniques of preparation (citrate, EDTA and heparin), and sera have been used to determine the specificity.
No false reactivity due to the method of specimen preparation has been observed.
Frozen specimens have also been tested to check whether this interferes with the performance of the test. No interference was observed on clean and particle free samples.
Samples derived from patients with different viral (HCV and HAV) and non viral pathologies of the liver that may interfere with the test were examined.
No cross reaction were observed.
The Performance Evaluation study conducted in a qualified external reference center on more than 500 samples has provided a value > 98% .

4. Precision
It has been calculated on two samples examined in 16 replicate in three different runs on three lots.
The values found were as follows:

HBE.CE: lot # 0103

Negative Control (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.030	0.027	0.032	0.029
Std.Deviation	0.002	0.002	0.003	0.002
CV %	7.4	8.2	7.9	7.8

PEI 1 U/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.569	0.559	0.575	0.568
Std.Deviation	0.027	0.029	0.028	0.028
CV %	4.7	5.3	4.9	4.9
S/Co	4.4	4.4	4.4	4.4

HBE.CE: lot # 0103/2

Negative Control (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.033	0.031	0.030	0.032
Std.Deviation	0.003	0.003	0.002	0.003
CV %	7.9	8.5	7.4	8.0

PEI 1 U/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.565	0.573	0.568	0.569
Std.Deviation	0.026	0.025	0.024	0.025
CV %	4.7	4.3	4.2	4.4
S/Co	4.2	4.4	4.4	4.3

HBE.CE: lot # 0303

Negative Control (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.029	0.034	0.038	0.034
Std.Deviation	0.003	0.003	0.004	0.003
CV %	9.7	9.8	9.2	9.6

PEI 1 U/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.579	0.573	0.564	0.572
Std.Deviation	0.023	0.028	0.025	0.025
CV %	4.1	4.8	4.5	4.5
S/Co	4.5	4.3	4.1	4.3

B) HBe Antibody

1. Limit of detection

The limit of detection of the assay has been calculated by means of the International Standard for HBeAb, supplied by Paul Erlich Institute (PEI).

The data obtained by examining the limit of detection on three lots is reported in the table below.

HBE.CE Lot ID	PEI U/ml HBeAb
0103	0.25
0103/2	0.25
0303	0.25

In addition the preparation Accurun # 52, produced by Boston Biomedica Inc., USA, has been tested, upon dilution in FCS. Results are reported for three lots of products.

Accurun 52 (Co/S)

HBE.CE Lot ID	1 x	2 x	4 x	8 x	16x
0103	1.0	0.8	0.6	0.4	0.4
0103/2	1.0	0.8	0.6	0.5	0.4
0303	1.0	0.8	0.6	0.4	0.4

2. Diagnostic sensitivity:

The diagnostic sensitivity has been tested on panels of samples classified positive for HBeAb by a US FDA approved kit.

Positive samples were collected from different HBV pathologies bearing anti HBeAg antibody reactivity.

An overall value > 98% has been found in the study conducted on a total number of more than 200 samples.

Moreover the Panel of Seroconversion code PHM 935B, produced by BBI, was examined.

Data are reported below and compared with those reported by BBI for two other commercial products.

Sample ID	HBE.CE Co/S	Abbott EIA Co/S	Sorin EIA Co/S
21	0.4	0.4	0.5
22	0.4	0.5	0.6
23	0.4	0.6	0.5
24	0.4	0.5	0.6
25	0.4	0.6	0.5
26	0.5	0.6	0.6
27	0.6	0.8	0.7
28	0.7	0.9	0.7
29	0.6	0.9	0.7
30	0.8	1.0	0.9
31	1.0	1.3	1.1
32	1.0	1.2	1.0

Finally the Performance Panel code PHJ 201, produced by BBI, was tested. Data are reported below and compared with those reported by BBI for another commercial product.

Member	PEI U/ml	HBE.CE	Sorin EIA
1	-	0.3	0.5
2	-	0.2	0.5
3	-	0.2	0.5
4	-	0.2	0.5
5	-	0.3	0.6
6	-	0.3	0.6
7	-	0.2	0.4
8	-	0.2	0.4
9	-	0.2	0.5
10	-	1.9	0.6
11	-	0.3	0.5
12	-	0.4	0.9
13	2	4.4	9.1
14	1	3.8	2.9
15	< 1	1.0	1.5
16	> 50	4.3	120.9
17	< 1	1.0	1.0
18	5	5.6	21.8
19	1	2.7	6.4
20	11	5.0	47.3
21	2	1.9	10.0
22	26	28.1	90.7
23	-	0.3	0.5
24	< 1	0.8	1.3
25	50	28.1	167.4

3. Diagnostic specificity:

The clinical specificity has been determined as described before for HBeAg.

The Performance Evaluation study conducted in a qualified external reference center on more than 500 samples has provided a value > 98% .

4. Precision:

It has been calculated on two samples examined in 16 replicate in three different runs on three lots.

The values found were as follows:

HBE.CE: lot # 0103

Negative Control (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	2.484	2.420	2.471	2.458
Std.Deviation	0.129	0.160	0.142	0.144
CV %	5.2	6.6	5.7	5.9

PEI 0.25 U/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.867	0.800	0.878	0.848
Std.Deviation	0.043	0.060	0.050	0.051
CV %	5.0	7.5	5.7	6.1
Co/S	1.0	1.0	1.0	1.0

HBE.CE: lot # 0103/2

Negative Control (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	2.316	2.361	2.413	2.363
Std.Deviation	0.127	0.144	0.146	0.139
CV %	5.5	6.1	6.0	5.9

PEI 0.25 U/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.767	0.793	0.785	0.781
Std.Deviation	0.041	0.050	0.046	0.046
CV %	5.4	6.3	5.8	5.8
Co/S	1.0	1.0	1.0	1.0

HBE.CE: lot #0303

Negative Control (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	2.334	2.415	2.437	2.395
Std.Deviation	0.146	0.155	0.158	0.153
CV %	6.3	6.4	6.5	6.4

PEI 0.25 U/ml (N = 16)

Mean values	1st run	2nd run	3rd run	Average value
OD 450nm	0.850	0.867	0.876	0.864
Std.Deviation	0.052	0.051	0.048	0.050
CV %	6.1	5.9	5.5	5.8
Co/S	0.9	1.0	1.0	1.0

Important note:
The performance data have been obtained proceeding as the reading step described in the section M, point 14.

S. LIMITATIONS

Frozen samples containing fibrin particles or aggregates may generate false positive results.
Bacterial contamination or heat inactivation of the specimen may affect the absorbance values of the samples with consequent alteration of the level of the analyte.
This test is suitable only for testing single samples and not pooled ones.
Diagnosis of an infectious disease should not be established on the basis of a single test result. The patient's clinical history, symptomatology, as well as other diagnostic data should be considered.

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All the IVD Products manufactured by the company are under the control of a certified Quality Management System approved by an EC Notified Body. Each lot is submitted to a quality control and released into the market only if conforming with the EC technical specifications and acceptance criteria.

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Monobind Inc.
Lake Forest, CA 92630, USA

AccuBind
ELISA Microwells

β-Human Chorionic Gonadotropin (hCG) Test System

Product Code: 825-300

1.0 INTRODUCTION

Intended Use: The Quantitative Determination of Chorionic Gonadotropin (hCG) Concentration in Human Serum by a Microplate Enzyme Immunoassay, Colorimetric

2.0 SUMMARY AND EXPLANATION OF THE TEST

Human chorionic gonadotropin (hCG) concentration increases dramatically in blood and urine during normal pregnancy. hCG is secreted by placental tissue, beginning with the primitive trophoblast, almost from the time of implantation, and serves to support the corpus luteum during the early weeks of pregnancy. hCG or hCG similar glycoproteins can also be produced by a wide variety of trophoblastic and nontrophoblastic tumors. The measurement of hCG, by assay systems with suitable sensitivity and specificity has proven great value in the detection of pregnancy and the diagnosis *of early* pregnancy disorders.

According to the literature, hCG is detectable as early as 10 days after ovulation, reaching 100 mIU/ml by the first missed period. At the time for the next ovulation, the hCG level is 200 mIU/ml (approximately 28 days after conception).[1] A peak of 50,000 or even 100,000 mIU/ml is attained by the third month, then a gradual decline is observed.[2,3]

In this method, hCG calibrator, patient specimen or control is first added to a streptavidin coated well. Biotinylated monoclonal and enzyme labeled antibodies (directed against distinct and different epitopes of hCG) are added and the reactants mixed. Reaction between the various hCG antibodies and native hCG forms a sandwich complex that binds with the streptavidin coated to the well.

After the completion of the required incubation period, the enzyme-chorionic gonadotropin antibody bound conjugate is separated from the unbound enzyme-chorionic gonadotropin conjugate by aspiration or decantation. The activity of the enzyme present on the surface of the well is quantitated by reaction with a suitable substrate to produce color.

The employment of several serum references of known chorionic gonadotropin levels permits construction of a dose response curve of activity and concentration. From comparison to the dose response curve, an unknown specimen's activity can be correlated with chorionic gonadotropin concentration.

3.0 PRINCIPLE

Immunoenzymometric assay (TYPE 3):
The essential reagents required for an immunoenzymometric assay include high affinity and specificity antibodies (enzyme and immobilized), with different and distinct epitope recognition, **in excess,** and native antigen. In this procedure, the immobilization takes place during the assay at the surface of a microplate well through the interaction of streptavidin coated on the well and exogenously added biotinylated monoclonal anti-hCG antibody. Upon mixing monoclonal biotinylated antibody, the enzyme-labeled antibody and a serum containing the native antigen, reaction results between the native antigen and the antibodies without competition or steric hindrance to form a soluble sandwich complex. The interaction is illustrated by the following equation:

$$^{Enz}Ab_{(x\text{-}hCG)} + Ag_{hCG} + {}^{Btn}Ab_{(m)} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} {}^{Enz}Ab_{(m)} - Ag_{hCG} - {}^{Btn}Ab_{(m)}$$

$^{Btn}Ab_{(m)}$ = Biotinylated Monoclonal Antibody (Excess Quantity)
Ag_{hCG} = Native Antigen (Variable Quantity)
$^{Enz}Ab_{(x\text{-}hCG)}$ = Enzyme labeled Antibody (Excess Quantity)
$^{Enz}Ab_{(hCG)}$ - Ag_{hCG} - $^{Btn}Ab_{(m)}$ = Ag-Antibodies Sandwich complex
k_a = Rate Constant of Association
k_{-a} = Rate Constant of Dissociation

Simultaneously, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antibody. This interaction is illustrated below:
$^{Enz}Ab_{(x\text{-}hCG)}$-$Ag_{hCG}$-$^{Btn}Ab_{(m)}$ + $Streptavidin_{CW}$ ⇒ immobilized complex
$Streptavidin_{CW}$ = Streptavidin immobolized on well
Immobilized complex = sandwich complex bound to the well

After equilibrium is attained, the antibody-bound fraction is separated from unbound antigen by decantation or aspiration. The enzyme activity in the antibody-bound fraction is directly proportional to the native antigen concentration. By utilizing several different serum references of known antigen values, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

4.0 REAGENTS

Materials Provided:

A. hCG Calibrators – 1 ml/vial - Icons A-F
Six (6) vials of references for hCG Antigen at levels of 0(**A**), 5(**B**), 25(**C**), 50(**D**), 100(**E**) and 250(**F**) mIU/ml. Store at 2-8°C. A preservative has been added.
Note: The calibrators, human serum based, were calibrated using a reference preparation, which was assayed against the WHO 3rd IS (75/537).

B. hCG Enzyme Reagent – 13 ml/vial - Icon
One (1) vial containing enzyme labeled affinity purified antibody, biotinylated monoclonal mouse IgG in buffer, dye, and preservative. Store at 2-8°C.

C. Streptavidin Coated Plate – 96 wells – Icon ⇓
One 96-well microplate coated with streptavidin and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

D. Wash Solution Concentrate – 20 ml/vial - Icon
One (1) vial containing a surfactant in buffered saline. A preservative has been added. Store at 2-8°C.

E. Substrate A – 7ml/vial - Icon S^A
One (1) vial containing tetramethylbenzidine (TMB) in buffer. Store at 2-8°C.

F. Substrate B – 7ml/vial - Icon S^B
One (1) vial containing hydrogen peroxide (H_2O_2) in buffer. Store at 2-8°C.

G. Stop Solution – 8ml/vial - Icon
One (1) vial containing a strong acid (1N HCl). Store at 2-8°C.

H. Product Instructions.

Note 1: Do not use reagents beyond the kit expiration date.
Note 2: Avoid extended exposure to heat and light. **Opened reagents are stable for sixty (60) days when stored at 2-8°C. Kit and component stability are identified on the label.**
Note 3: Above reagents are for a single 96-well microplate

4.1 Required But Not Provided:
1. Pipette(s) capable of delivering 0.025 and 0.050ml (25 & 50µl) volumes with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100 and 0.350ml (100 & 350µl) volumes with a precision of better than 1.5%.
3. Microplate washers or a squeeze bottle (optional).
4. Microplate Reader with 450nm and 620nm wavelength absorbance capability.
5. Absorbent Paper for blotting the microplate wells.
6. Plastic wrap or microplate cover for incubation steps.
7. Vacuum aspirator (optional) for wash steps.
8. Timer.
9. Quality control materials

5.0 PRECAUTIONS

For In Vitro Diagnostic Use
Not for Internal or External Use in Humans or Animals

All products that contain human serum have been found to be non-reactive for Hepatitis B Surface Antigen, HIV 1&2 and HCV Antibodies by FDA licensed reagents. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Edition, 1988, HHS Publication No. (CDC) 88-8395.

Safe Disposal of kit components must be according to local regulatory and statutory requirement.

6.0 SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood, serum in type and the usual precautions in the collection of venipuncture samples should be observed. For accurate comparison to established normal values, a fasting morning serum sample should be obtained. The blood should be collected in a plain redtop venipuncture tube without additives or anti-coagulants. Allow the blood to clot. Centrifuge the specimen to separate the serum from the cells.

In patients receiving therapy with high biotin doses (i.e. >5mg/day), no sample should be taken until at least 8 hours after the last biotin administration, preferably overnight to ensure fasting sample.

Samples may be refrigerated at 2-8°C for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20°C for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.05 ml (50µl) of the specimen is required.

7.0 QUALITY CONTROL

Each laboratory should assay controls at levels in the low, normal and elevated range for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

8.0 REAGENT PREPARATION

1. **Wash Buffer**
Dilute contents of wash concentrate to 1000ml with distilled or deionized water in a suitable storage container. Store diluted buffer at 2-30°C for up to 60 days.
2. **Working Substrate Solution** – Stable for one year
Pour the contents of the amber vial labeled Solution 'A' into the clear vial labeled Solution 'B'. Place the yellow cap on the clear vial for easy identification. Mix and label accordingly. Store at 2 - 8°C.

Note1: Do not use the working substrate if it looks blue.
Note 2: Do not use reagents that are contaminated or have bacteria growth.

9.0 TEST PROCEDURE

Before proceeding with the assay, bring all reagents, serum reference calibrators and controls to room temperature (20-27°C).
*****Test Procedure should be performed by a skilled individual or trained professional*****

1. Format the microplate wells for each serum reference calibrator, control and patient specimen to be assayed in duplicate. **Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C**
2. Pipette 0.025 ml (25µl) of the appropriate serum reference calibrator, control or specimen into the assigned well.
3. Add 0.100 ml (100µl) of hCG-Enzyme Reagent to all wells.
4. Swirl the microplate gently for 20-30 seconds to mix and cover.
5. Incubate 60 minutes at room temperature.
6. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.
7. Add 0.350ml (350µl) of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.**
8. Add 0.100 ml (100µl) of working substrate solution to all wells (see Reagent Preparation Section). **Always add reagents in the same order to minimize reaction time differences between wells**
DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION
9. Incubate at room temperature for fifteen (15) minutes.
10. Add 0.050ml (50µl) of stop solution to each well and gently mix for 15-20 seconds). **Always add reagents in the same order to minimize reaction time differences between wells**
11. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) in a microplate reader. **The results should be read within thirty (30) minutes of adding the stop solution.**

10.0 CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of Human chorionic gonadotropin (hCG) in unknown specimens.
1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the absorbance for each duplicate serum reference versus the corresponding hCG concentration in mIU/ml on linear graph paper (do not average the duplicates of the serum references before plotting).
3. Draw the best-fit curve through the plotted points.
4. To determine the concentration of hCG for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in mIU/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance (1.745) intersects the dose response curve at (157 mIU/ml) hCG concentration (See Figure 1).

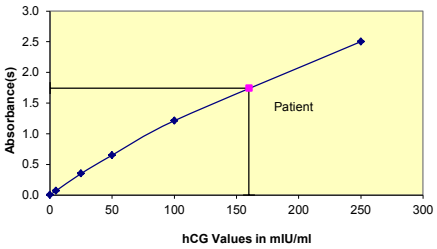
Note: Computer data reduction software designed for ELISA assays may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained.

EXAMPLE 1

Sample I.D.	Well Number	Abs (A)	Mean Abs (B)	Value (mIU/ml)
Cal A	A1	0.002	0.004	0
	B1	0.005		
Cal B	C1	0.073	0.071	5
	D1	0.069		
Cal C	E1	0.340	0.350	25
	F1	0.360		
Cal D	G1	0.637	0.650	50
	H1	0.663		
Cal E	A2	1.223	1.212	100
	B2	1.199		
Cal F	C2	2.518	2.502	250
	D2	2.486		
Ctrl 1	E2	0.075	0.076	**5.8**
	F2	0.077		
Ctrl 2	G2	0.280	0.290	**21.9**
	H2	0.301		
Patient	A3	1.736	1.745	**157**
	B3	1.754		

*The data presented in Example 1 and Figure 1 are for illustration only and **should not** be used in lieu of a dose response curve prepared with each assay.

Figure 1



11.0 Q.C. PARAMETERS

In order for the assay results to be considered valid the following criteria should be met:

1. The absorbance (OD) of calibrator 'F' should be ≥ 1.3.
2. Four out of six quality control pools should be within the established ranges.

12.0 RISK ANALYSIS

The MSDS and Risk Analysis Form for this product are available on request from Monobind Inc.

12.1 Assay Performance

1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. The addition of substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the substrate and stop solution should be added in the same sequence to eliminate any time-deviation during reaction.
6. Plate readers measure vertically. Do not touch the bottom of the wells.
7. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
8. Use components from the same lot. No intermixing of reagents from different batches.
9. Patient specimens with hCG concentrations above 250 mIU/ml may be diluted with normal male serum (hCG < 1 mIU/ml) and re-assayed. The sample's concentration is obtained by multiplying the result by the dilution factor.
10. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from Monobind IFU may yield inaccurate results.
11. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.
12. It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.
13. Risk Analysis- as required by CE Mark IVD Directive 98/79/EC - for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com.

12.2 Interpretation

1. ***Measurements and interpretation of results must be performed by a skilled individual or trained professional.***
2. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.
3. The reagents for the test system have been formulated to eliminate maximal interference; however, potential interaction between rare serum specimens and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for all kinds of immunoassays (Boscato LM, Stuart MC. 'Heterophilic antibodies: a problem for all immunoassays' Clin. Chem. 1988:3427-33). For diagnostic purposes, the results from this assay should be in combination with clinical examination, patient history and all other clinical findings.For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
4. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, Monobind shall have no liability.
5. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.
6. False positive results may occur in the presence of a wide variety of trophoblastic and nontrophoblastic tumors that secrete hCG. Therefore, the possibility of an hCG secreting neoplasia should be eliminated prior to diagnosing pregnancy.
7. Also, false positive results may be seen when assaying specimens from individuals taking the drugs Pergonal* and Clomid**. Additionally Pergonal will often be followed with an injection of hCG.
8. Spontaneous microabortions and ectopic pregnancies will tend to have values which are lower than expected during a normal pregnancy while somewhat higher values are often seen in multiple pregnancies.[5,6,7]
9. Following therapeutic abortion, detectable hCG may persist for as long as three to four weeks. The disappearance rate of hCG, after spontaneous abortion, will vary depending upon the quantity of viable residual trophoblast.[4,5,6,7]
10. **A hCG value alone is not of diagnostic value** and should only be used in conjunction with other clinical manifestations (observations) and diagnostic procedures.

*Pegonal is a registered trademark of Serono Laboratories, Inc.
**Clomid is a registered trademark of Merriell-National Laboratories

13.0 EXPECTED RANGES OF VALUES

A study of an apparent normal adult population was undertaken to determine expected values for the HCG AccuBind® ELISA Test System. The mean (X) values, standard deviations (**σ**) and expected ranges (±2**σ**) are presented in Table 1.

TABLE I
Expected Values for the hCG ELISA Test System
(In mIU/ml - 3rd IS 75/537)

Number	25
Mean	2.9
Standard Deviation	1.4
Expected Ranges (±2σ)	0.1 - 5.7

Expected levels for hCG during normal pregnancy (3) are listed in Table 2.

TABLE 2
Expected Values for hCG levels (3rd IS 75/537)
during normal pregnancy (in mIU/ml)

1st week	**10 - 30**
2nd week	30 - 100
3rd week	100 - 1000
4th week	1,000 -10,000
2nd & 3rd month	30,000 - 100,000
2nd trimester	10,000 - 30,000
3rd trimester	5,000 - 15,000

Values for hCG for a normal, healthy population and pregnant women, during gestation cycle, are given in Table 3. The values depicted below represent limited in house studies in concordance with published literature.[8,9,10]

TABLE 3
Median Values during Gestation.

Gestation (Week)	hCG (IU/ml)
15	40.88
16	33.87
17	28.71
18	26.74
19	18.76
20	19.24
21	23.46

It is important to keep in mind that establishment of a range of values which can be expected to be found by a given method for a population of "normal"-persons is dependent upon a multiplicity of factors: the specificity of the method, the population tested and the precision of the method in the hands of the analyst. For these reasons each laboratory should depend upon the range of expected values established by the Manufacturer only until an in-house range can be determined by the analysts using the method with a population indigenous to the area in which the laboratory is located.

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

The within and between assay precisions of the hCG AccuBind® ELISA were determined by analyses on three different levels of control sera. The number (N), mean value (X), standard deviation (σ) and coefficient of variation (C.V.) for each of these control sera are presented in Table 4 and Table 5.

TABLE 4
Within Assay Precision (Values in mIU/ml)

Sample	N	X	σ	C.V.
Level 1	20	4.4	0.22	4.9%
Level 2	20	18.7	0.75	4.0%
Level 3	20	214.8	14.59	6.8%

TABLE 5
Between Assay Precision* (Values in mIU/ml)

Sample	N	X	σ	C.V.
Level 1	20	5.4	0.52	9.6%
Level 2	20	22.4	1.97	8.8%
Level 3	20	213.1	15.16	7.1%

*As measured in ten experiments in duplicate.

14.2 Sensitivity

The hCG AccuBind® ELISA test system has a sensitivity of 0.003 mIU/well. This is equivalent to a sample containing 0.102 mIU/ml hCG concentration. The analytical sensitivity (detection limit) was ascertained by deteremining the variability of the '0 mIU/ml' calibrator and using the 2σ (95% certainty) statistic to calculate the minimum dose.

14.3 Accuracy

This hCG AccuBind® ELISA test system was compared with a reference radioimmunoassay. Biological specimens from normal and pregnant populations were assayed. The total number of such specimens was 110. The least square regression equation and the correlation coefficient were computed for the hCG ELISA in comparison with the reference method. The data obtained is displayed below.

TABLE 6

Method	Mean (x)	Least Square Regression Analysis	Correlation Coefficient
Monobind	14.8	y = 0.081 + 0.93(x)	0.989
Reference	15.1		

Only slight amounts of bias between the hCG ELISA method and the reference method are indicated by the closeness of the mean values. The least square regression equation and correlation coefficient indicates excellent method agreement.

14.4 Specificity

The cross-reactivity of the hCG AccuBind® ELISA to selected substances was evaluated by adding the interfering substance to a serum matrix at various concentrations, The cross-reactivity was calculated by deriving a ratio between dose of interfering substance to dose of chorionic gonadotropin needed to produce the same absorbance.

Substance	Cross Reactivity	Concentration
Chorionic Gonadotropin (hCG)	1.0000	----
β-hCG subunit	< 0.0001	1000ng/ml
Follitropin (FSH)	< 0.0001	1000ng/ml
Lutropin Hormone (LH)	< 0.0001	1000ng/ml
hrotropin (TSH)	< 0.0001	1000ng/ml

14.5 Hook Effect

The test shows no hook effect up to concentrations of > 150,000 mIU/ml.

15.0 REFERENCES

1. Kosasa TS, "Measurement of Human Chorionic Gonadotropin", *Journal of Reproductive Medicine,* 26, 201-6 (1981).
2. Danzer H, Braunstein GD, et al, "Maternal Serum Human Chorionic Gonadotropic Concentrations and Fetal Sex Predictions", *Fertility and Sterility,* 34, 336-40 (1980).
3. Braunstein G.D., et al., "Serum Human Chorionic Gonadotropin Levels through Normal Pregnancy", *American Journal of Obstetrics and Gynecology* 126, pg. 678-81 (1976).
4. Goldstein DP, and Kosasa TS, "The Subunit Radioimmunoassay for HCG Clinical Application", *Gynecology, 6,* 145-84 (1975).
5. Batzer F, "Hormonal Evaluation of Early Pregnancy", *Fertility and Sterility,* 34, 1-12 (1980).
6. Braunstein GD, et al, "First-Trimester Chorionic Gonadotropin Measurements as an Aid to the Diagnosis of Early Pregnancy Disorders", *American Journal of Obstetrics and Gynecology,* 131, 25-32 (1978).
7. Lenton E, Neal L and Sulaiman R, "Plasma Concentrations of Human Gonadotropin from the time of Implantation until the Second Week of Pregnancy ", *Fertility and Sterility,* 37, 773-78 (1982).
8. Canick JA, Rish S. 'The accuracy of assigned risks in maternal serum screening', *Prenatal Diagnosis*; 18:413-415 (1998).
9. NIH State-of-the Science Conference Statement on Management of Menopause-Related Symptoms. NIH Consensus State Sci Statements. Mar 21-23; 22(1), 1-38 (2005).
10. Tietz NW, ED: *Clinical Guide to Laboratory Tests* 3rd Ed, Philadelphia, WA Saunders Co (1995).

Revision: 5 Date: 2021-Sep-23 DCO: 1509
MP825 Product Code: *825-300*

Size		96(A)	192(B)
Reagent (fill)	A)	1ml set	1ml set
	B)	1 (13ml)	2 (13ml)
	C)	1 plate	2 plates
	D)	1 (20ml)	1 (20ml)
	E)	1 (7ml)	2 (7ml)
	F)	1 (7ml)	2 (7ml)
	G)	1 (8ml)	2 (8ml)

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Please visit our website to learn more about our products and services.

Glossary of Symbols
(EN 980/ISO 15223)

IVD In Vitro - Diagnostic Medical Device
Temperature Limitation Storage Condition (2-8° C)
Consult Instructions for Use
REF Catalogue Number
Contains Sufficient Test for Σ
LOT Batch Code
Used By (Expiration Day)
Date of Manufacturer
Manufacturer
EC REP Authorized Rep in European Country
CE European Conformity

Monobind Inc.
Lake Forest, CA 92630, USA

AccuBind
ELISA Microwells

Follicle Stimulating Hormone (FSH) Test System

Product Code: 425-300

1.0 INTRODUCTION

Intended Use: The Quantitative Determination of Follicle Stimulating Hormone Concentration in Human Serum by a Microplate Enzyme Immunoassay, Colorimetric.

2.0 SUMMARY AND EXPLANATION OF THE TEST

Follicle Stimulating hormone (FSH) is a glycoprotein consisting of two subunits with an approximate molecular mass of 35,500 daltons. The α-subunit is similar to other pituitary hormones [luteinizing stimulating hormone (LH), thyroid stimulating hormone (TSH) and chorionic gonadotropin (CG)] while the β-subunit is unique. The β-subunit confers the biological activity to the molecule. Stimulation by gonadotropin-releasing hormone (GnRH) causes release of FSH, as well as LH, from the pituitary and is transported by the blood to their sites of action, the testes or ovary.

In men, FSH acts on the Sertoli cells of the testis, stimulating the synthesis of inhibin, which appears to specifically inhibit further FSH secretion, and androgen-binding protein. Thus, it indirectly supports spermatogenesis. In women, FSH acts on the granulosa cells of the ovary, stimulating steroidogensis. All ovulatory menstrual cycles have a characteristic pattern of FSH, as well as LH, secretion. The menstrual cycle is divided into a follicular phase and a luteal phase by the midcycle surge of the gonadotropins (LH and FSH). As the follicular phase progresses, FSH concentration decreases. Near the time ovulation occurs, about midcycle, FSH peaks (lesser in magnitude than LH) to its highest level.

The clinical usefulness of the measurement of Follicle Stimulating hormone (FSH) in ascertaining the homeostasis of fertility regulation via the hypothalamic - pituitary - gonadal axis has been well established.[1,2]

In this method, FSH calibrator, patient specimen or control is first added to a streptavidin coated well. Biotinylated monoclonal and enzyme labeled antibodies (directed against distinct and different epitopes of FSH) are added and the reactants mixed. Reaction between the various FSH antibodies and native FSH forms a sandwich complex that binds with the streptavidin coated to the well.

After the completion of the required incubation period, the enzyme-Follicle Stimulating Hormone antibody bound conjugate is separated from the unbound enzyme-follicle stimulating hormone conjugate by aspiration or decantation. The activity of the enzyme present on the surface of the well is quantitated by reaction with a suitable substrate to produce color.

The employment of several serum references of known Follicle Stimulating Hormone levels permits construction of a dose response curve of activity and concentration. From comparison to the dose response curve, an unknown specimen's activity can be correlated with Follicle Stimulating Hormone concentration.

3.0 PRINCIPLE

Immunoenzymometric assay (TYPE 3):
The essential reagents required for an immunoenzymometric assay include high affinity and specificity antibodies (enzyme and immobilized), with different and distinct epitope recognition, **in excess,** and native antigen. In this procedure, the immobilization takes place during the assay at the surface of a microplate well through the interaction of streptavidin coated on the well and exogenously added biotinylated monoclonal anti-FSH antibody.
Upon mixing monoclonal biotinylated antibody, the enzyme-labeled antibody and a serum containing the native antigen, reaction results between the native antigen and the antibodies without competition or steric hindrance to form a soluble sandwich complex. The interaction is illustrated by the following equation:

$$^{Enz}Ab_{(p)} + Ag_{FSH} + {}^{Btn}Ab_{(m)} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} {}^{Enz}Ab_{(p)} - Ag_{FSH} - {}^{Btn}Ab_{(m)}$$

$^{Btn}Ab_{(m)}$ = Biotinylated Monoclonal Antibody (Excess Quantity)
Ag_{FSH} = Native Antigen (Variable Quantity)
$^{Enz}Ab_{(p)}$ = Enzyme labeled Antibody (Excess Quantity)
$^{Enz}Ab_{(p)} - Ag_{FSH} - {}^{Btn}Ab_{(m)}$ = Antigen-Antibodies Sandwich Complex
k_a = Rate Constant of Association
k_{-a} = Rate Constant of Dissociation

Simultaneously, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antibody. This interaction is illustrated below:
$^{Enz}Ab_{(p)} - Ag_{FSH} - {}^{Btn}Ab_{(m)} + Streptavidin_{C.W.} \Rightarrow$ Immobilized complex
$Streptavidin_{C.W.}$ = Streptavidin immobolized on well
Immobilized complex = sandwich complex bound to the solid surface

After equilibrium is attained, the antibody-bound fraction is separated from unbound antigen by decantation or aspiration. The enzyme activity in the antibody-bound fraction is directly proportional to the native antigen concentration. By utilizing several different serum references of known antigen values, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

4.0 REAGENTS

Materials Provided:

A. FSH Calibrators – 1 ml/vial - Icons A-F
Six (6) vials of references for FSH Antigen at levels of 0(**A**), 5(**B**), 10(**C**), 25(**D**), 50**E**) and 100(**F**) mIU/ml. Store at 2-8°C. A preservative has been added.
Note: The calibrators, human serum based, were calibrated using a reference preparation, which was assayed against the WHO 2nd IRP (78/549).

B. FSH Enzyme Reagent – 13 ml/vial - Icon Ⓔ
One (1) vial-containing enzyme labeled antibody, biotinylated monoclonal mouse IgG in buffer, dye, and preservative. Store at 2-8°C.

C. Streptavidin Coated Plate – 96 wells - Icon ⇓
One 96-well microplate coated with streptavidin and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

D. Wash Solution Concentrate – 20 ml/vial - Icon
One (1) vial containing a surfactant in buffered saline. A preservative has been added. Store at 2-8°C.

E. Substrate A – 7.0ml/vial - Icon S[A]
One (1) vial containing tetramethylbenzidine (TMB) in buffer. Store at 2-8°C.

F. Substrate B – 7.0ml/vial - Icon S[B]
One (1) vial containing hydrogen peroxide (H_2O_2) in buffer. Store at 2-8°C.

G. Stop Solution – 8ml/vial - Icon STOP
One (1) vial containing a strong acid (1N HCl). Store at 2-8°C.

H. Product Instructions.

Note 1: Do not use reagents beyond the kit expiration date.
Note 2: Avoid extended exposure to heat and light. **Opened reagents are stable for sixty (60) days when stored at 2-8°C. Kit and component stability are identified on the label.**
Note 3: Above reagents are for a single 96-well microplate**.**

4.1 Required But Not Provided:
1. Pipette capable of delivering 0.050ml (50µl) and 0.100ml (100µl) volumes with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100ml (100µl) and 0.350ml (350µl) volumes with a precision of better than 1.5%.
3. Microplate washers or a squeeze bottle (optional).
4. Microplate Reader with 450nm and 620nm wavelength absorbance capability.
5. Absorbent Paper for blotting the microplate wells.
6. Plastic wrap or microplate cover for incubation steps.
7. Vacuum aspirator (optional) for wash steps.
8. Timer.
9. Quality control materials

5.0 PRECAUTIONS

For In Vitro Diagnostic Use
Not for Internal or External Use in Humans or Animals

All products that contain human serum have been found to be non-reactive for Hepatitis B Surface Antigen, HIV 1&2 and HCV Antibodies by FDA licensed reagents. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Edition, 1988, HHS Publication No. (CDC) 88-8395.

Safe Disposal of kit components must be according to local regulatory and statutory requirement.

6.0 SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood, serum in type and the usual precautions in the collection of venipuncture samples should be observed. For accurate comparison to established normal values, a fasting morning serum sample should be obtained. The blood should be collected in a plain redtop venipuncture tube without additives or anti-coagulants. Allow the blood to clot. Centrifuge the specimen to separate the serum from the cells.

In patients receiving therapy with high biotin doses (i.e. >5mg/day), no sample should be taken until at least 8 hours after the last biotin administration, preferably overnight to ensure fasting sample.

Samples may be refrigerated at 2-8°C for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20°C for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.100ml (100µl) of the specimen is required.

7.0 QUALITY CONTROL

Each laboratory should assay controls at levels in the low, normal and elevated range for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

8.0 REAGENT PREPARATION

1. **Wash Buffer**
   Dilute contents of wash concentrate to 1000ml with distilled or deionized water in a suitable storage container. Store at 2-30°C for up to 60 days.
2. **Working Substrate Solution** – Stable for one year
   Pour the contents of the amber vial labeled Solution 'A' into the clear vial labeled Solution 'B'. Place the yellow cap on the clear vial for easy identification. Mix and label accordingly. Store at 2 - 8°C.

Note1 : Do not use the working substrate if it looks blue.
Note 2: Do not use reagents that are contaminated or have bacteria growth.

9.0 TEST PROCEDURE

Before proceeding with the assay, bring all reagents, serum reference calibrators and controls to room temperature (20-27°C).
*****Test Procedure should be performed by a skilled individual or trained professional*****

1. Format the microplate wells for each serum reference calibrator, control and patient specimen to be assayed in duplicate. **Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.**
2. Pipette 0.050 ml (50µl) of the appropriate serum reference calibrator, control or specimen into the assigned well.
3. Add 0.100 ml (100µl) of FSH-Enzyme Reagent solution to all wells.
4. Swirl the microplate gently for 20-30 seconds to mix and cover.
5. Incubate 60 minutes at room temperature.
6. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.
7. Add 350µl of wash buffer (see Reagent Preparation Section) decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.**
8. Add 0.100 ml (100µl) of working substrate solution to all wells (see Reagent Preparation Section). **Always add reagents in the same order to minimize reaction time differences between wells**

DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION

9. Incubate at room temperature for fifteen (15) minutes.
10. 10.Add 0.050ml (50µl) of stop solution to each well and gently mix for 15-20 seconds). **Always add reagents in the same order to minimize reaction time differences between wells**
11. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) in a microplate reader. **The results should be read within thirty (30) minutes of adding the stop solution.**

10.0 CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of follicle stimulating hormone in unknown specimens.
1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the absorbance for each duplicate serum reference versus the corresponding FSH concentration in mIU/ml on linear graph paper (do not average the duplicates of the serum references before plotting).
3. Draw the best-fit curve through the plotted points.
4. To determine the concentration of FSH for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in mIU/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance (1.214) intersects the dose response curve at 43.2mIU/ml FSH concentration (See Figure 1).

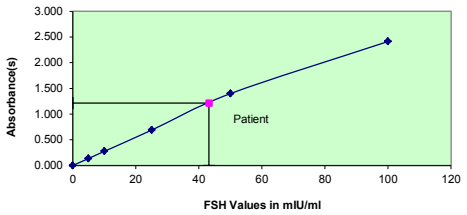
Note: Computer data reduction software designed for ELISA assays may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained.

*The data presented in Example 1 and Figure 1 are for illustration only and **should not** be used in lieu of a dose response curve prepared with each assay.

EXAMPLE 1

Sample I.D.	Well Number	Abs (A)	Mean Abs (B)	Value (mIU/ml)
Cal A	A1	0.001	0.001	0
	B1	0.001		
Cal B	C1	0.146	0.139	5
	D1	0.133		
Cal C	E1	0.276	0.277	10
	F1	0.278		
Cal D	G1	0.680	0.689	25
	H1	0.698		
Cal E	A2	1.444	1.399	50
	B2	1.354		
Cal F	C2	2.471	2.412	100
	D2	2.354		
Ctrl 1	E2	0.162	0.157	***5.6***
	F2	0.152		
Ctrl 2	G2	0.545	0.546	***19.9***
	H2	0.547		
Patient	A3	1.173	1.214	***43.2***
	B3	1.255		

Figure 1



11.0 Q.C. PARAMETERS

In order for the assay results to be considered valid the following criteria should be met:

1. The absorbance (OD) of calibrator F should be ≥ 1.3
2. Four out of six quality control pools should be within the established ranges.

12.0 RISK ANALYSIS

The MSDS and Risk Analysis Form for this product is available on request from Monobind Inc.

12.1 Assay Performance

1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. The addition of substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the substrate and stop solution should be added in the same sequence to eliminate any time-deviation during reaction.
6. Plate readers measure vertically. Do not touch the bottom of the wells.
7. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
8. Use components from the same lot. No intermixing of reagents from different batches.
9. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from Monobind IFU may yield inaccurate results.
10. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.
11. It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.
12. Risk Analysis- as required by CE Mark IVD Directive 98/79/EC - for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com.

12.2 Interpretation

1. ***Measurements and interpretation of results must be performed by a skilled individual or trained professional.***
2. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.
3. The reagents for the test system have been formulated to eliminate maximal interference; however, potential interaction between rare serum specimens and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for all kinds of immunoassays (Boscato LM, Stuart MC. 'Heterophilic antibodies: a problem for all immunoassays' Clin. Chem. 1988:3427-33). For diagnostic purposes, the results from this assay should be in combination with clinical examination, patient history and all other clinical findings.For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
4. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, Monobind shall have no liability.
5. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.
6. FSH is suppressed by estrogen but in woman taking oral contraceptives the level may be low or normal. Excessive dieting and weight loss may lead to low gonadotropin concentrations.
7. Follicle Stimulating Hormones are dependent upon diverse factors other than pituitary homeostasis. Thus, the determination alone is not sufficient to assess clinical status.

13.0 EXPECTED RANGES OF VALUES

A study of an apparent normal adult population was undertaken to determine expected values for the FSH Accubind® ELISA Test System. The expected values are presented in Table 1.

TABLE 1
Expected Values for the FSH Accubind® ELISA Test System (in mIU/ml 2nd IRP 78/549)

Women			
Follicular phase	3.0	--	12.0
Midcycle	8.0	--	22.0
Luteal phase	2.0	--	12.0
Postmenopausal	35.0	--	151.0
Men			
1.0	--	14.0	

It is important to keep in mind that establishment of a range of values which can be expected to be found by a given method for a population of "normal"-persons is dependent upon a multiplicity of factors: the specificity of the method, the population tested and the precision of the method in the hands of the analyst. For these reasons each laboratory should depend upon the range of expected values established by the manufacturer only until an in-house range can be determined by the analysts using the method with a population indigenous to the area in which the laboratory is located.

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

The within and between assay precisions of the FSH Accubid® ELISA test system were determined by analyses on three different levels of control sera. The number (N), mean value (X), standard deviation (σ) and coefficient of variation (C.V) for each of these control sera are presented in Table 2 and Table 3.

TABLE 2
Within Assay Precision (Values in mIU/ml)

Sample	N	X	σ	C.V.
Level 1	20	5.0	0.25	5.4%
Level 2	20	25.0	0.94	3.8%
Level 3	20	40.6	1.64	4.0%

TABLE 3
Between Assay Precision* (Values in mIU/ml)

Sample	N	X	σ	C.V.
Level 1	20	4.7	0.42	9.0%
Level 2	20	23.1	1.99	8.6%
Level 3	20	37.8	3.2	8.4%

*As measured in ten experiments in duplicate.

14.2 Sensitivity

The Follicle Stimulating Hormone procedure has a sensitivity of 0.006 mIU/well. This is equivalent to a sample containing 0.134mIU/ml FSH concentration.The sensitivity (detection limit) was ascertained by determining the variability of the '0 mIU/ml' calibrator and using the 2σ (95% certainty) statistic to calculate the minimum dose.

14.3 Accuracy

This FSH Accubind® ELISA test system was compared with a reference radioimmunoassay. Biological specimens from low, normal, and elevated concentrations were assayed. The total number of such specimens was 106. The least square regression equation and the correlation coefficient were computed for the FSH Accubind® ELISA test system in comparison with the reference method. The data obtained is displayed in Table 4.

TABLE 4

Method	Mean (x)	Least Square Regression Analysis	Correlation Coefficient
Monobind	17.4	y = 0.98(x) – 1.7	0.978
Reference	19.5		

Only slight amounts of bias between the FSH Accubind® ELISA test method and the reference method are indicated by the closeness of the mean values. The least square regression equation and correlation coefficient indicates excellent method agreement.

14.4 Specificity

The cross-reactivity of the FSH Accubind® ELISA test system to selected substances was evaluated by adding the interfering substance to a serum matrix at various concentrations. The cross-reactivity was calculated by deriving a ratio between dose of interfering substance to dose of Follicle Stimulating Hormone needed to produce the same absorbance.

Substance	Cross Reactivity	Concentration
Follitropin (FSH)	1.0000	--
Lutropin Hormone (hLH)	< 0.0001	1000ng/ml
Chorionic Gonadotropin (hCG)	< 0.0001	1000ng/ml
Thyrotropin (TSH)	< 0.0001	1000ng/ml

15.0 REFERENCES

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Revision: 4 Date: 2019-Jul-16 DCO: 1353
MP425 Cat #: *425-300*

Size		96(A)	192(B)
Reagent (fill)	A)	1ml set	1ml set
	B)	1 (13ml)	2 (13ml)
	C)	1 plate	2 plates
	D)	1 (20ml)	1 (20ml)
	E)	1 (7ml)	2 (7ml)
	F)	1 (7ml)	2 (7ml)
	G)	1 (8ml)	2 (8ml)

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www.cepartner4u.eu

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Glossary of Symbols
(EN 980/ISO 15223)

IVD — In Vitro - Diagnostic Medical Device
2° 8° — Temperature Limitation Storage Condition (2-8°C)
Consult Instructions for Use
REF — Catalogue Number
Σ — Contains Sufficient Test for Σ
LOT — Batch Code
Used By (Expiration Day)
Date of Manufacturer
Manufacturer
EC REP — Authorized Rep in European Country
CE — European Conformity

ВЕКТОР БЕСТ

IgA общий-ИФА-БЕСТ

A-8666

Набор реагентов
для иммуноферментного определения
концентрации общего иммуноглобулина
класса А в сыворотке крови

ИНСТРУКЦИЯ ПО ПРИМЕНЕНИЮ

Утверждена 14.08.2018

1. НАЗНАЧЕНИЕ

1.1. Набор реагентов для иммуноферментного определения концентрации общего иммуноглобулина класса А в сыворотке крови «IgA общий-ИФА-БЕСТ» (далее по тексту – набор) предназначен для определения концентрации общего иммуноглобулина класса А ($IgA_{общ}$) в сыворотке крови человека методом твердофазного иммуноферментного анализа.

1.2. Набор рассчитан на проведение анализа в дублях 41 неизвестного, 6 калибровочных и 1 контрольного образцов (всего 96 определений при использовании всех стрипов планшета).

2. ХАРАКТЕРИСТИКА НАБОРА

2.1. Принцип метода

Метод определения основан на двухстадийном «сэндвич»-варианте твердофазного иммуноферментного анализа с применением моноклональных антител к IgA.

На первой стадии калибровочные образцы с известной концентрацией $IgA_{общ}$ и анализируемые образцы инкубируются в лунках стрипированного планшета с иммобилизованными моноклональными антителами (МКАТ) к альфа-цепям IgA. На второй стадии связавшийся в лунках IgAобщ обрабатывают конъюгатом МКАТ к легким (лямбда и каппа) цепям иммуноглобулинов человека с пероксидазой.

Образовавшиеся иммунные комплексы «иммобилизованные МКАТ – IgA – конъюгат» выявляют ферментативной реакцией с раствором тетраметилбензидина. Степень окрашивания пропорциональна концентрации $IgA_{общ}$ в анализируемом образце. После измерения величины оптической плотности раствора в лунках на основании калибровочного графика рассчитывается концентрация $IgA_{общ}$ в анализируемых образцах.

2.2. Состав набора

В состав набора входят:

- планшет разборный (12 восьмилуночных стрипов) с иммобилизованными на внутренней поверхности лунок моноклональными антителами к альфа-цепям IgA человека, готовый для использования – 1 шт.;
- калибровочные образцы, содержащие известные количества $IgA_{общ}$ – 0; 17,5; 35; 75; 150; 300 Ед/мл (0; 0,25; 0,49; 1,05; 2,1 и 4,2 мг/мл); аттестованные относительно WHO International Standard Immunoglobulins G, A and M, human serum, NIBSC 67/086; концентрации $IgA_{общ}$ в калибровочных образцах могут несколько отличаться от указанных величин, точные величины указаны на этикетках флаконов, готовые для использования – 6 флаконов (по 0,5 мл);
- контрольный образец на основе инактивированной сыворотки крови человека с известным содержанием $IgA_{общ}$, аттестованный относительно WHO International Standard Immunoglobulins G, A and

M, human serum, NIBSC 67/086; готовый для использования – 1 флакон (0,5 мл);
– конъюгат моноклональных антител к легким (лямбда и каппа) цепям иммуноглобулинов человека с пероксидазой хрена, готовый для использования – 1 флакон (13 мл);
– раствор для разведения сывороток (РРС), концентрат – 1 флакон (28 мл);
– концентрат фосфатно-солевого буферного раствора с твином (ФСБ-Т×25) – 1 флакон (28 мл);
– раствор тетраметилбензидина плюс (раствор ТМБ плюс), готовый для использования – 1 флакон (13 мл);
– стоп-реагент, готовый для использования – 1 флакон (12 мл).
– пленка для заклеивания планшета – 2 шт.;
– ванночка для реагента – 2 шт.;
– наконечники для пипетки на 5–200 мкл – 16 шт.;
– планшет для предварительного разведения исследуемых образцов – 1 шт.

Принадлежности:
– трафарет для построения калибровочного графика – 1 шт.

3. АНАЛИТИЧЕСКИЕ ХАРАКТЕРИСТИКИ

3.1. Специфичность. В наборе «IgA общий-ИФА-БЕСТ» используются моноклональные антитела, обладающие высокой специфичностью к альфа-цепям IgA. Перекрестного связывания с IgG, IgM, IgE или альбумином в физиологических концентрациях не наблюдалось.

3.2. «Хук»-эффект при использовании набора реагентов не зафиксирован. Оптическая плотность образцов сыворотки крови с концентрацией $IgA_{общ}$ до 1000 Ед/мл всегда превышала оптическую плотность калибровочного образца с максимальной концентрацией $IgA_{общ}$.

3.3.* Воспроизводимость. Коэффициент вариации результатов определения концентрации $IgA_{общ}$ в лунках, содержащих контрольный образец, не превышает 8%.

3.4.* Линейность. Данный аналитический параметр проверяется тестом на «линейность» - отклонение от расчетной величины концентрации $IgA_{общ}$ при разведении калибровочных образцов, содержащих 300, 150, 75, 35 Ед/мл в 2 раза. Процент «линейности» составляет: 90–110 %.

3.5.* Точность. Данный аналитический параметр проверяется тестом на «открытие» – соответствие измеренной концентрации $IgA_{общ}$ расчетному значению в пробе, полученной путем смешивания равных объемов контрольного образца и калибровочного образца с концентрацией $IgA_{общ}$ 35 Ед/мл (0,49 мг/мл). Процент «открытия» составляет 90–110%.

3.6.* Чувствительность. Минимально определяемая концентрация $IgA_{общ}$, рассчитанная на основании среднего арифметического значения оптической плотности калибровочного

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* по ГОСТ Р 51352-2013.

образца B_0 (с концентрацией $IgA_{общ}$ 0 Ед/мл) плюс 2σ (σ – среднее квадратичное отклонение от среднего арифметического значения B_0) не превышает 1,5 Ед/мл (0,021 мг/мл).

3.7. Клиническая проверка. Концентрация $IgA_{общ}$, измеренная в сыворотке крови, у условно здоровых доноров, находилась в диапазоне 20÷285 Ед/мл (см. также с. 29).

3.8. Рекомендуется в каждой лаборатории при использовании набора уточнить значения концентрации $IgA_{общ}$, соответствующие нормальным для данного региона у обследуемого контингента людей.

4. МЕРЫ ПРЕДОСТОРОЖНОСТИ

4.1. Потенциальный риск применения набора – класс 2а (Приказ МЗ РФ от 06.06.2012 № 4н).

4.2. Все компоненты набора являются нетоксичными. Стоп-реагент обладает раздражающим действием. Избегать разбрызгивания и попадания на кожу и слизистые. В случае попадания стоп-реагента на кожу и слизистые необходимо промыть пораженный участок большим количеством проточной воды.

4.3. При работе с исследуемыми образцами следует соблюдать меры предосторожности, принятые при работе с потенциально инфекционным материалом. Основные правила работы изложены в «Инструкции по мерам профилактики распространения инфекционных заболе-

ваний при работе в клинико-диагностических лабораториях лечебно-профилактических учреждений», утвержденной Минздравом СССР 17 января 1991 г. и в методических указаниях МУ 287-113 «Методические указания по дезинфекции, предстерилизационной очистке и стерилизации изделий медицинского назначения», утв. департаментом госсанэпиднадзора Минздрава РФ от 30.12.1998.

4.4. При работе с набором следует надевать одноразовые резиновые или пластиковые перчатки, так как образцы сыворотки крови человека следует рассматривать как потенциально инфекционные, способные длительное время сохранять и передавать ВИЧ, вирусы гепатита или возбудителей других инфекций.

4.5. Лабораторная посуда и оборудование, которые используются в работе с набором, должны быть соответствующим образом промаркированы и храниться отдельно.

4.6. Запрещается прием пищи, использование косметических средств и курение в помещениях, предназначенных для работы с наборами.

4.7. Для дезинфекции посуды и материалов, контактировавших с исследуемыми и контрольными образцами, рекомендуем использовать дезинфицирующие средства, не оказывающие негативного воздействия на качество ИФА, не содержащие активный кислород и хлор, например, комбинированные средства на основе

ЧАС, спиртов, третичных аминов. Использование дезинфицирующих средств, содержащих активный кислород и хлор (H_2O_2, деохлор, хлорамин), приводит к серьезному искажению результатов.

4.8. При использовании набора образуются отходы классов А, Б и Г, которые классифицируются и уничтожаются (утилизируются) в соответствии с СанПиН 2.1.7.2790-10 «Санитарно-эпидемиологические требования к обращению с медицинскими отходами». Дезинфекцию наборов реагентов следует проводить по МУ 287-113 «Методические указания по дезинфекции, предстерилизационной очистке и стерилизации изделий медицинского назначения».

5. ОБОРУДОВАНИЕ И МАТЕРИАЛЫ, НЕОБХОДИМЫЕ ПРИ РАБОТЕ С НАБОРОМ:

– спектрофотометр вертикального сканирования, позволяющий проводить измерения оптической плотности растворов в лунках стрипов при основной длине волны 450 нм и длине волны сравнения в диапазоне 620–655 нм; допускается измерение только при длине волны 450 нм;
– шейкер термостатируемый орбитального типа, позволяющий производить встряхивание при температуре 37±1°С и 400–800 об/мин;
– микроцентрифуга, позволяющая центрифугировать при 1500–2000 об/мин;
– промывочное устройство для планшетов;
– холодильник бытовой;

– пипетки полуавтоматические одноканальные с переменным или фиксированным объемом со сменными наконечниками, позволяющие отбирать объемы жидкости от 5 до 5000 мкл;
– пипетка полуавтоматическая многоканальная со сменными наконечниками, позволяющая отбирать объемы жидкостей от 5 до 350 мкл;
– флаконы стеклянные вместимостью 15 мл;
– цилиндр мерный вместимостью 1000 мл;
– вода дистиллированная;
– перчатки медицинские диагностические одноразовые;
– бумага фильтровальная лабораторная;
– дезинфицирующий раствор.

6. АНАЛИЗИРУЕМЫЕ ОБРАЗЦЫ

6.1. Для проведения анализа не следует использовать гемолизованную, мутную сыворотку крови.

6.2. Образцы сыворотки крови можно хранить при температуре от 2 до 8°С не более 48 часов, при температуре минус 20°С (и ниже) не более 3 месяцев. Повторное замораживание и размораживание образцов сыворотки крови не допускается. После размораживания образцы следует тщательно перемешать.

6.3. Образцы сывороток крови, содержащие осадок, необходимо очистить центрифугированием при 1500 об/мин в течение 5 мин при температуре от 18 до 25°С.

7. ПРОВЕДЕНИЕ АНАЛИЗА

ПОДГОТОВКА РЕАГЕНТОВ

7.1. Перед проведением анализа компоненты набора и исследуемые образцы следует выдержать при температуре от 18 до 25°С не менее 30 мин.

7.2. Подготовка планшета

Вскрыть пакет выше замка и установить на рамку необходимое для проведения анализа количество стрипов. Использовать в течение 1 часа после установки. Оставшиеся стрипы немедленно поместить вновь в пакет с влагопоглотителем, удалить из него воздух, плотно закрыть замок.

Хранить при температуре от 2 до 8°С в течение всего срока годности набора.

7.3. Приготовление промывочного раствора

Раствор готовится из концентрата фосфатно-солевого буферного раствора. При выпадении осадка солей в концентрате необходимо прогреть его при температуре 30–40°С до полного растворения осадка.

Внести в мерный цилиндр необходимое количество концентрата фосфатно-солевого буферного раствора с твином (ФСБ-Т×25) и добавить соответствующее количество дистиллированной воды.

В таблице приведен расход реагента в зависимости от количества используемых стрипов.

Приготовленный промывочный раствор можно хранить при температуре от 2 до 8°С не более 5 сут.

7.4. Приготовление рабочего раствора для разведения сывороток

Приготовить за 30 мин до начала постановки анализа.

При выпадении осадка солей в концентрате РРС необходимо прогреть его при температуре 30–40°С до полного растворения осадка.

Внести в мерный цилиндр необходимое количество концентрата раствора для разведения сывороток и добавить соответствующее количество дистиллированной воды.

В таблице приведен расход реагента в зависимости от количества используемых стрипов.

Приготовленный рабочий раствор для разведения сывороток можно хранить при температуре от 2 до 8°С не более 3 сут.

7.5. Приготовление калибровочных образцов и контрольного образца

Калибровочные образцы и контрольный образец готовы к использованию и не требуют дополнительного разведения. Перед использованием флаконы встряхнуть или центрифугировать на микроцентрифуге так, чтобы капли растворов со стенок и крышки опустились на дно. Затем содержимое флаконов тщательно

Таблица расхода компонентов набора реагентов

Кол-во одновременно используемых стрипов	Промывочный раствор		Рабочий раствор для разведения сывороток		Конъюгат, мл	Раствор ТМБ плюс, мл
	ФСБ-Т×25, мл	Дистиллированная вода, мл	РРС, концентрат, мл	Дистиллированная вода, мл		
2	4,0	до 100	4,0	до 100	2,0	2,0
3	6,0	до 150	6,0	до 150	3,0	3,0
4	8,0	до 200	8,0	до 200	4,0	4,0
5	10,0	до 250	10,0	до 250	5,0	5,0
6	12,0	до 300	12,0	до 300	6,0	6,0
7	14,0	до 350	14,0	до 350	7,0	7,0
8	16,0	до 400	16,0	до 400	8,0	8,0
9	18,0	до 450	18,0	до 450	9,0	9,0
10	20,0	до 500	20,0	до 500	10,0	10,0
11	22,0	до 550	22,0	до 550	11,0	11,0
12	24,0	до 600	24,0	до 600	12,0	12,0

перемешать на вортексе или пипетированием, избегая образования пены.

Калибровочные образцы и контрольный образец после вскрытия можно хранить в плотно закрытых флаконах при температуре от 2 до 8°С в течение всего срока годности набора.

7.6. Приготовление рабочего разведения анализируемых образцов сыворотки крови

Готовится в стеклянных заранее промаркированных флаконах за 5–10 мин до начала постановки анализа.

В чистый флакон с 10 мл рабочего раствора для разведения сывороток (см. п. 7.4) добавить 10 мкл исследуемой сыворотки и тщательно перемешать. Таким образом, рабочее разведение сыворотки составляет 1000 раз*.

Использовать в течение 30 мин после приготовления.

7.7. Подготовка конъюгата.

Конъюгат готов к использованию.

Необходимое количество конъюгата отобрать в чистый флакон или ванночку для реагента.

Оставшийся после проведения ИФА конъюгат утилизировать ***(не сливать во флакон с исходным конъюгатом).***

В таблице приведен расход реагента в зависимости от количества используемых стрипов.

Конъюгат после вскрытия можно хранить в плотно закрытом флаконе при температуре от 2 до 8°С в течение всего срока годности набора.

7.8. Подготовка раствора тетраметилбензидина плюс.

* См. также раздел «Дополнительная информация для потребителей», п. 2

Раствор ТМБ плюс готов к использованию.

Необходимое количество раствора ТМБ плюс отобрать в чистый флакон или ванночку для реагента.

Оставшийся после проведения ИФА раствор ТМБ плюс утилизировать ***(не сливать во флакон с исходным раствором ТМБ плюс).***

Необходимо исключить воздействие прямого света на раствор ТМБ плюс.

Раствор ТМБ плюс после вскрытия можно хранить в плотно закрытом флаконе при температуре от 2 до 8°С в течение всего срока годности набора.

В таблице приведен расход реагента в зависимости от количества используемых стрипов.

7.9. Стоп-реагент готов к использованию.

После вскрытия стоп-реагент можно хранить в плотно закрытом флаконе при температуре от 2 до 8°С в течение всего срока годности набора.

ПРОВЕДЕНИЕ ИФА

7.10. Внести во все лунки по 100 мкл рабочего раствора для разведения сывороток (см п. 7.4).

Внести в соответствующие лунки в дублях, начиная с верхних лунок первых двух стрипов, по 20 мкл каждого калибровочного образца. В следующую пару лунок внести по 20 мкл контрольного образца. В остальные лунки внести в дублях по 20 мкл анализируемых образцов сы-

воротки крови в рабочем разведении (см п. 7.6), каждый раз меняя наконечник.

Время внесения образцов не должно превышать 10 мин при использовании всех лунок планшета.

7.11. Планшет заклеить пленкой и инкубировать в течение 20 мин при встряхивании на термостатируемом шейкере при температуре 37±1°С и 700 об/мин.

7.12. По окончании инкубации снять липкую пленку и удалить ее в сосуд с дезинфицирующим раствором. Содержимое лунок удалить отсасыванием в сосуд с дезинфицирующим раствором и промыть, добавляя во все лунки по 350 мкл промывочного раствора. Процесс промывки повторить еще 4 раза. Общее количество отмывок равно 5. Время между заполнением и опорожнением лунок должно быть не менее 30 сек. Необходимо следить за полным опорожнением лунок после каждого цикла отмывки. Затем удалить остатки жидкости из лунок, постукивая планшетом в перевернутом положении по фильтровальной бумаге.

7.13. Внести во все лунки планшета по 100 мкл конъюгата (см п. 7.7).

Для внесения конъюгата использовать ванночку для реагента и одноразовые наконечники, входящие в состав набора.

7.14. Планшет заклеить пленкой и инкубировать в течение 20 мин при встряхивании на

термостатируемом шейкере при температуре 37±1°С и 700 об/мин.

7.15. По окончании инкубации удалить содержимое лунок и промыть планшет, как это указано в п. 7.12.

7.16. Внести во все лунки по 100 мкл раствора ТМБ плюс (см п. 7.8) и инкубировать в защищенном от света месте в течение 15 мин при температуре от 18 до 25°С.

Для внесения раствора ТМБ плюс использовать ванночку для реагента и одноразовые наконечники, входящие в состав набора.

7.17. Внести во все лунки с той же скоростью и в той же последовательности, как и раствор ТМБ плюс, по 100 мкл стоп-реагента, при этом содержимое лунок окрашивается в желтый цвет.

8. РЕГИСТРАЦИЯ РЕЗУЛЬТАТОВ

Измерить величину оптической плотности растворов в лунках стрипов на спектрофотометре вертикального сканирования в двухволновом режиме: основной фильтр – 450 нм, референс-фильтр в диапазоне 620–655 нм; допускается измерение только с фильтром 450 нм. Измерение проводить через 2–3 мин после остановки реакции.

Время между остановкой реакции и измерением оптической плотности не должно превышать 10 мин.

9. УЧЕТ РЕЗУЛЬТАТОВ

9.1. Вычислить средние арифметические значения оптической плотности для каждой пары лунок, содержащих калибровочные образцы.

9.2. Построить в линейных координатах калибровочный график зависимости среднего арифметического значения оптической плотности (ед. опт. плотн.) от концентрации $IgA_{общ}$ в калибровочных образцах (Ед/мл или мг/мл).

9.3. Определить концентрацию $IgA_{общ}$ в контрольном образце и анализируемых образцах по калибровочному графику. Вычислить среднее арифметическое значение концентрации для каждой пары лунок, содержащих анализируемые образцы.

9.4. Если при проведении анализа использовали разведение сыворотки в 1000 раз (базовое разведение для данного набора), то найденное по графику количество $IgA_{общ}$ соответствует концентрации $IgA_{общ}$ в анализируемом образце в Ед/мл (мг/мл). Если использовали другое разведение образца, то найденное по графику количество $IgA_{общ}$ пересчитывают с учетом дополнительного разведения, также получая в результате концентрацию $IgA_{общ}$ в Ед/мл (мг/мл).

Если значение оптической плотности анализируемого образца превышает значение ОП для калибровочного образца 300 Ед/мл (4,2 мг/мл), то данный образец анализируют повторно после до-

полнительного разведения в 2 раза, полученный результат умножить на 2.

10. УСЛОВИЯ ТРАНСПОРТИРОВАНИЯ, ХРАНЕНИЯ И ПРИМЕНЕНИЯ НАБОРА

10.1. Транспортировать изделия следует транспортом всех видов в крытых транспортных средствах в соответствии с правилами перевозок, действующими на транспорте данного вида, при температуре от 2 до 8°С. Допускается транспортирование при температуре до 25°С не более 10 суток.

10.2. Хранение набора в упаковке предприятия-изготовителя должно осуществляться при температуре от 2 до 8°С в течение всего срока годности в холодильных камерах или холодильниках, обеспечивающих регламентированный температурный режим с ежедневной регистрацией температуры.

10.3. Срок годности набора – 12 месяцев со дня выпуска. Не допускается применение наборов по истечении срока их годности.

10.4. Дробное использование набора может быть реализовано в течение всего срока годности.

В случае дробного использования набора:

– неиспользованные стрипы можно хранить в плотно закрытом пакете при температуре от 2 до 8°С в течение всего срока годности набора;
– калибровочные образцы, контрольный образец и конъюгат после вскрытия можно хранить в плот-

но закрытых флаконах при температуре от 2 до 8°С в течение всего срока годности набора;
– концентрат фосфатно-солевого буферного раствора с твином, концентрат раствора для разведения сывороток; раствор ТМБ плюс и стоп-реагент после вскрытия можно хранить в плотно закрытых флаконах при температуре от 2 до 8°С в течение всего срока годности набора;
– рабочий раствор для разведения сывороток можно хранить при температуре от 2 до 8°С не более 3 сут;
– промывочный раствор можно хранить при температуре от 2 до 8°С не более 5 сут.

10.5. Построение калибровочного графика необходимо проводить для каждого независимого эксперимента, рекомендуется также каждый раз определять концентрацию $IgA_{общ}$ в контрольном образце.

10.6. Для перевода результатов измерений концентрации общего иммуноглобулина класса А из Ед/мл в мг/мл следует использовать коэффициент пересчета 0,014 (1 Ед/мл $IgA_{общ}$ = 0,014 мг/мл $IgA_{общ}$).

10.7. При постановке ИФА нельзя использовать компоненты из наборов разных серий или смешивать их при приготовлении растворов, кроме неспецифических компонентов (ФСБ-Т×25, раствор ТМБ плюс, стоп-реагент), которые взаимозаменяемы во всех наборах АО «Вектор-Бест».

10.8. Для получения надежных результатов необходимо строгое соблюдение инструкции по применению набора.

11. ГАРАНТИЙНЫЕ ОБЯЗАТЕЛЬСТВА

11.1. Производитель гарантирует соответствие выпускаемых изделий требованиям нормативной и технической документации.

Безопасность и качество изделия гарантируются в течение всего срока годности.

11.2. Производитель отвечает за недостатки изделия, за исключением дефектов, возникших вследствие нарушения правил пользования, условий транспортирования и хранения, либо действия третьих лиц, либо непреодолимой силы.

11.3. Производитель обязуется за свой счет заменить изделие, технические и функциональные характеристики (потребительские свойства) которого не соответствуют нормативной и технической документации, если указанные недостатки явились следствием скрытого дефекта материалов или некачественного изготовления изделия производителем.

По вопросам, касающимся качества набора «IgA общий-ИФА-БЕСТ»,
следует обращаться в АО «Вектор-Бест»
по адресу:
630559, Новосибирская область,
Новосибирский район,
п. Кольцово, а/я 121,
тел. (383) 363-20-60, 227-75-43,
тел./факс (383) 363-35-55,
E-mail: vbobtk@vector-best.ru

ДОПОЛНИТЕЛЬНАЯ ИНФОРМАЦИЯ ДЛЯ ПОТРЕБИТЕЛЕЙ

Набор предназначен для профессионального применения в клинической лабораторной диагностике обученным персоналом.

Требования безопасности к медицинским лабораториям приведены в ГОСТ Р 52905-2007.

Все реагенты наборов, содержащие в своем составе материалы человеческого происхождения, инактивированы.

При динамическом наблюдении пациента для получения результатов, адекватно отражающих изменение концентрации IgA в крови, необходимо использовать наборы реагентов одного наименования (одного предприятия-изготовителя).

1. Обеспечение получения правильных результатов анализа

Достоверность и воспроизводимость результатов анализа зависят от выполнения следующих основных правил:

– не проводите ИФА в присутствии паров кислот, щелочей, альдегидов или пыли, которые могут влиять на ферментативную активность конъюгатов;

– ферментативная реакция чувствительна к присутствию ионов металлов, поэтому не допускайте контактов каких-либо металлических предметов с конъюгатом и раствором ТМБ;

– избегайте загрязнения компонентов набора микроорганизмами и химическими примесями, для этого используйте в работе чистую посуду и чистые одноразовые наконечники для каждого реагента, контроля, образца;

– рабочие поверхности столов, оборудования обрабатывайте 70% этиловым спиртом (не допускается использование перекиси водорода, хлорсодержащих растворов);

– никогда не используйте одну и ту же емкость для конъюгата и раствора ТМБ;

– перед отбором ТМБ из флакона необходимо обрабатывать конус пипетки (внутреннюю и внешнюю поверхности) сначала дистиллированной водой, а затем 70% этиловым спиртом, так как малейшее загрязнение пипеток конъюгатом может привести к контаминации всего содержимого флакона с ТМБ;

– если допущена ошибка при внесении анализируемого образца, нельзя, опорожнив эту лунку, вносить в нее новый образец; такая лунка бракуется.

Качество промывки лунок планшета играет важную роль для получения правильных результатов анализа:

– Для аспирации анализируемых образцов и последующей промывки рекомендуется использовать автоматическое или ручное промывочное устройство.

– Не допускайте высыхания лунок планшета в перерыве между завершением промывки и внесением реагентов.

– Добивайтесь полного заполнения и опорожнения всех лунок планшета в процессе промывки. Недостаточная аспирация жидкости в процессе промывки может привести к понижению чувствительности и специфичности анализа.

– Следите за состоянием промывочного устройства – регулярно (1 раз в неделю) обрабатывайте шланги и емкости 70% этиловым спиртом.

– Для предотвращения засорения игл промывочного устройства в конце рабочего дня обязательно выполните процедуру ополаскивания системы подачи жидкости дистиллированной водой.

2. Рекомендации по подготовке анализируемых образцов

Вместо одноступенчатого (п. 7.6.) допустимо проводить двухступенчатое разведение сывороток

с использованием планшета для предварительного разведения исследуемых образцов. Для этого, в каждую лунку планшета для предварительного разведения внести по 310 мкл рабочего раствора для разведения сывороток. Далее в одну из лунок, например, А-1, добавить 10 мкл исследуемой сыворотки, сменить использованный наконечник пипетки на новый и затем с его помощью тщательно перемешать содержимое лунки (5–6 круговых движений, во время которых следует 3–4 раза набрать и опорожнить наконечник), избегая образования пены. После этого из лунки отобрать 10 мкл, внести в соседнюю лунку, например, А-2, и таким же образом тщательно перемешать (для этой операции также желательно использовать новый чистый наконечник пипетки). В лунке А-2 получаем рабочее разведение сыворотки 1000 раз. Аналогично развести и другие исследуемые сыворотки (например, в лунках В-1 и В-2, С-1 и т.д.):

– 310 мкл рабочего раствора для разведения сывороток + 10 мкл исследуемого образца → предварительное разведение образца в 32 раза;

– 310 мкл рабочего раствора для разведения сывороток + 10 мкл образца после предварительного разведения → рабочее разведение образца в 1000 раз.

Внимание! *Точность приготовления разведений определяет качество постановки теста!*

При исследовании не сыворотки, а других биологических жидкостей, степень разведения исследуемых образцов следует заранее подобрать

Таблица 2

Абсолютные значения уровней содержания иммуноглобулинов в различных биологических жидкостях у здоровых лиц (M±δ)

(Тотолян А.А. Современные подходы к диагностике иммунопатологических состояний. Мед.иммунология, 1999 ,т.1 №1-2, с. 75-108)

Биологические жидкости	Содержание иммуноглобулинов классов:				
	IgA, г/л	IgM, г/л	IgG, г/л	sIgA, г/л	IgE, кЕ/л
ЦСЖ	0,006±0,0013	0,0049±0,001	0,037±0,004	н/опр	0±0
Слюна	0,069±0,028	0,055±0,011	0,042±0,017	0,768±0,275	н/опр
Назальный смыв	0,014±0,006	0,025±0,017	0,042±0,017	0,071±0,022	0±0
Ларингеальный секрет	0,071±0,022	0,063±0,044	0,085±0,044	1,31±1,87	н/опр
Слезная жидкость	0,165±0,02	0,038±0,008	0,185±0,06	н/опр	н/опр
Эякулят	1,01±0,67	0,9±0,46	0,51±0,2	2,21±1,01	0±0
Сыворотка крови	2,15±0,85	1,63±0,46	12,3±2,97	0,79±0,22	50,0±12,5

***Примечание**: н/опр – данный показатель не определяли.*

Приведенные показатели можно использовать только как ориентировочные, и в каждой лаборатории рекомендуется вычислить собственные границы нормальных значений концентрации общего IgA в сыворотке крови.

опытным путем, используя как ориентир данные таблицы 2.

3. Условия правильности работы набора

Результаты анализа исследуемых образцов учитывать, если будут выполнены следующие условия:

– соотношение оптических плотностей калибровочных образцов: $ОП_0 < ОП_{17,5} < ОП_{35} < ОП_{75} < ОП_{150} < ОП_{300}$;

– $ОП_{300} \geq 1,0$ ед. опт. плотн. (о.е.);

– вычисленное по калибровочному графику значение концентрации $IgA_{общ}$ в контрольном образце попадает в пределы, указанные на этикетке флакона.

$ОП_0$, $ОП_{17,5}$, $ОП_{35}$, $ОП_{75}$, $ОП_{150}$ и $ОП_{300}$ – среднее значение оптической плотности калибровочных образов, содержащих 0; 17,5; 35; 75; 150 и 300 Ед/мл $IgA_{общ}$ соответственно.

4. Расчет результатов анализа

По результатам измерения вычислить среднее арифметическое значение оптической плотности (ОП) в лунках с анализируемыми образцами.

Построить в линейных координатах калибровочный график зависимости оптической плотности (ось ординат) от концентрации $IgA_{общ}$ (ось абсцисс) в калибровочных образцах. Для этого на прилагаемом трафарете для построения графика против концентрации каждого

калибровочного образца отложить соответствующее ей среднее значение оптической плотности. Последовательно соединить полученные точки отрезками прямых линий.

Пример калибровочного графика представлен на рисунке.

Определить содержание $IgA_{общ}$ в контрольном образце и в анализируемых образцах по калибровочному графику. Для этого на оси ординат отметить значение ОП анализируемого образца. Провести прямую линию, параллельно оси абсцисс, до пересечения с калибровочным графиком. От точки пересечения опустить

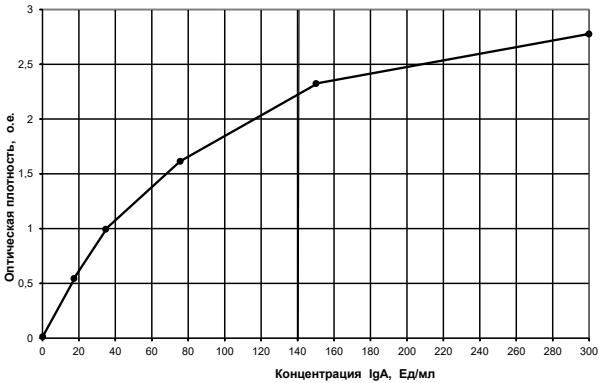

Рис. Зависимость оптической плотности от концентрации $IgA_{общ}$ в калибровочных образцах.

перпендикуляр на ось абсцисс. По полученной точке пересечения определить значение концентрации $IgA_{общ}$ в образце.

При использовании для расчетов концентраций компьютерного или встроенного в спектрофотометр программного обеспечения в настройках выбрать метод, соответствующий кусочно-линейной аппроксимации.

5. Диагностическая значимость

IgA, как и другие иммуноглобулины, относится к гуморальным факторам иммунитета. Карта гуморального иммунитета довольно индивидуальна, тем не менее, пределы нормальных физиологических концентраций достаточно хорошо очерчены.

По нашим данным, концентрация $IgA_{общ}$ в сыворотке крови клинически здоровых доноров (Новосибирская область, Алтайский край) в возрасте от 20 до 50 лет находится в пределах диапазона 57–285 Ед/мл (0,8–4,0мг/мл). У детей этого же региона в возрасте 1–15 лет концентрация $IgA_{общ}$ составила 20–200 Ед/мл. Эти значения в целом близки нормальным значениям концентрации IgA, приводимым в работе (Тотолян А.А., Марфичева Н.А., Тотолян Н.А. «Иммуноглобулины в клинической лабораторной диагностике», С-Пб, 1999.). Однако их можно использовать только как ориентировочные, поскольку диапазоны нормальных концентраций

IgA могут довольно существенно отличаться в зависимости от региона, возраста и некоторых др. причин. Известно также, что для использования в диагностике важнее знать не абсолютное значение концентрации общего иммуноглобулина, а его относительное отклонение от нормального местного, возрастного или, например, профессионального уровня. **В идеале, нормальные региональные уровни и по взрослым и по детям должны определяться каждой лабораторией самостоятельно!**

Уровень концентрации общего IgA в сыворотке крови новорожденных составляет около 1% от уровня взрослых. В возрасте 1–3 месяцев он обычно достигает 14%, 4–5 месяцев – 28%, 8–24 месяцев – 40%, 6 лет – 65%, 9 лет – 75%, 12–13 лет – 90–100% от уровня взрослого человека (15–45 лет).

Результаты определения концентрации общего сывороточного IgA могут быть с успехом использованы для дифференциальной диагностики целого ряда заболеваний (см. иммунограмму).

Более полную картину способно дать параллельное определение всех трех основных классов иммуноглобулинов – G, M и A, а также иммуноглобулина E.

Иммунограмма при некоторых заболеваниях

	IgG	IgA	IgM	IgE
Заболевания печени				
Острый инфекционный гепатит	+	N/+	N/++	N
Хронический персистирующий гепатит	N/+	N	N/+	N/+
Хронический агрессивный гепатит	++	+	N/++	N/+
Постгепатитный криптогенный цирроз	++	+	+	N/+
Первичный билиарный цирроз	N/+	N	+/++	N
Алкогольный цирроз	N/+	++	N/+	N
Болезни почек				
Острый пиелонефрит	N	N	+/++	N
Хронический пиелонефрит	+/++	N	+/++	N/+
Нефротический синдром	—	—	N/—	N/—
Инфекционные заболевания				
Острая инфекция	N	N	+/++	N
Хроническая инфекция	+/++	N/+	N/+	N/+
Системные ревматические заболевания				
Ревматоидный артрит	N/++	N/++	N/+	+/++
Системная красная волчанка	+	N	N/+	N/+
Склеродермия	N	N	N	N/+
Смешанные системные заболевания	N/+	N/+	N	N/+
Атопия, аллергические заболевания	N/+	N	N/—	+/++
Гельминтозы и др. паразитарные заболевания	N/+	N/+	N/+	+/++

N – нормальная регионально-возрастная концентрация иммуноглобулина (в пределах нормального диапазона от Nmin до Nmax)

\+ – повышенная концентрация иммуноглобулина (от Nmax до 1,3Nmax)

++ – сильно повышенная концентрация иммуноглобулина (более 1,3Nmax)

– – пониженная концентрация иммуноглобулина (ниже Nmin)

6. Краткая схема проведения ИФА для набора реагентов «IgA общий-ИФА-БЕСТ»

Использовать только после внимательного ознакомления с инструкцией!

Внести:	по 100 мкл рабочего раствора для разведения сывороток;
	по 20 мкл калибровочных и контрольного образцов в дублях в контрольные лунки;
	по 20 мкл разведенных анализируемых образцов в дублях в лунки для исследуемых образцов.
Инкубировать:	20 мин, 37°С, 700 об/мин.
Промыть:	промывочный раствор, 350 мкл, 5 раз.
Внести:	по 100 мкл конъюгата.
Инкубировать:	20 мин, 37°С, 700 об/мин.
Промыть:	промывочный раствор, 350 мкл, 5 раз.
Внести:	по 100 мкл раствора ТМБ плюс.
Инкубировать:	15 мин, 18–25°С, в темноте.
Внести:	по 100 мкл стоп-реагента.
Измерить:	ОП при 450 нм / референсная длина волны 620–655 нм.

7. Графические символы

REF	Номер по каталогу	IVD	Медицинское изделие для диагностики *in vitro*
Σ n	Содержимого достаточно для проведения n-количества тестов	NON STERILE	Не стерильно
LOT	Код партии		Температурный диапазон
	Изготовитель		Дата изготовления
	Использовать до …		Обратитесь к инструкции по применению
	Осторожно! Обратитесь к Инструкции по применению	YYYY-MM-DD YYYY-MM	Дата в формате Год-Месяц-День Год-Месяц

Консультацию специалиста по работе с набором можно получить по тел.: (383) 363-05-97.

12.11.18.

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www.vector-best.ru

Monobind Inc.
Lake Forest, CA 92630, USA

AccuBind
ELISA Microwells

Immunoglobulin E (IgE) Test System
Product Code: 2525-300

1.0 INTRODUCTION

Intended Use: The Quantitative Determination of Immunoglobulin E (IgE) Concentration in Human Serum by a Microplate Enzyme Immunoassay, Colorimetric

2.0 SUMMARY AND EXPLANATION OF THE TEST

Allergic reactions, which are becoming more widespread, are usually diagnosed on the basis of medical history and clinical symptoms. In vitro and in vivo testing, however, play a key role in confirming clinical suspicions and tailoring treatment. The measurement of immunoglobulin E (**IgE**) in serum is widely used in the diagnosis of allergic reactions and parasitic infections. Many allergies are caused by the immunoglobulins of subclass IgE acting as point of contact between the allergen and specialized cells. The IgE molecules (MW 200,000) bind to the surface of the mast cells and basophillic granulocytes. Subsequently the binding of allergen to cell-bound IgE causes these cells to release histamines and other vasoactive substances. The release of histamines in the body results initiates what is commonly known as an allergic reaction.

Before making any therapeutic determination it is important, however, to know whether the allergic reaction is IgE mediated or non-IgE mediated. Measurement of total IgE in serum sample, along with other supporting diagnostic information, can help to make that determination. Measurement of total circulating IgE may also be of value in the early detection of allergy in infants and as a means of predicting future atopic manifestations. Before deciding on any therapy it is important to take into consideration all the relevant clinical information as well as information supplied by specific allergy testing.

IgE levels show a slow increase during childhood, reaching adult levels in the second decade of life. In general, the total IgE levels increase with the allergies a person has and the number of times of exposure to the relevant allergens. Significant elevations may be seen in the sensitized individuals, but also in cases of myeloma, pulmonay aspergillosis, and during the active stages of parasitic infections.

In this method, IgE calibrator, patient specimen or control is first added to a streptavidin coated well. Biotinylated monoclonal antibody (specific for IgE) is added and the reactants mixed. Reaction between the IgE antibodies and native IgE forms complex that binds with the streptavidin coated to the well. The excess serum proteins are washed away via a wash step. Another enzyme labeled monoclonal antibody specific to IgE is added to the wells. The enzyme labeled antibody binds to the IgE already immobilized on the well through its binding with the biotinylated monoclonal antibody. Excess enzyme is washed off via a wash step. A color is generated by the addition of a substrate. The intensity of the color generation is directly proportional to the concentration of the IgE in the sample.

3.0 PRINCIPLE

Immunoenzymometric sequential assay (TYPE 4):
The essential reagents required for an immunoenzymometric assay include high affinity and specificity antibodies (enzyme and immobilized), with different and distinct epitope recognition, **in excess,** and native antigen. In this procedure, the immobilization takes place during the assay at the surface of a microplate well through the interaction of streptavidin coated on the well and exogenously added biotinylated monoclonal anti-IgE antibody.

Upon mixing monoclonal biotinylated antibody, and a serum containing the native antigen, reaction results between the native antigen and the antibody, forming an antibody-antigen complex. The interaction is illustrated by the following equation:

$$Ag_{(IgE)} + {}^{Btn}Ab_{(m)} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} Ag_{(IgE)} - {}^{Btn}Ab_{(m)}$$

${}^{Btn}Ab_{(m)}$ = Biotinylated Monoclonal Antibody (Excess Quantity)
$Ag_{I(gE)}$ = Native Antigen (Variable Quantity)
$Ag_{(IgE)} - {}^{Btn}Ab_{(m)}$ = Antigen-Antibody complex (Variable Quantity)
k_a = Rate Constant of Association
k_{-a} = Rate Constant of Disassociation

Simultaneously, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antibody. This interaction is illustrated below:
$Ag_{(IgE)} - {}^{Btn}Ab_{(m)}$ + $\underline{\text{Streptavidin}}_{C.W.} \Rightarrow \underline{\text{Immobilized complex}}$ (IC)
$\underline{\text{Streptavidin}}_{C.W.}$ = Streptavidin immobilized on well
$\underline{\text{Immobilized complex (IC)}}$ = Ag-Ab bound to the well

After a suitable incubation period, the antibody-antigen bound fraction is separated from unbound antigen by decantation or aspiration. Another antibody (directed at a different epitope) labeled with an enzyme is added. Another interaction occurs to form an enzyme labeled antibody-antigen-biotinylated-antibody complex on the surface of the wells. Excess enzyme is washed off via a wash step. A suitable substrate is added to produce color measurable with the use of a microplate spectrophotometer. The enzyme activity on the well is directly proportional to the native antigen concentration. By utilizing several different serum references of known antigen concentration, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

$$(IC) + {}^{Enz}Ab_{(x\text{-}IgE)} \underset{k_{-b}}{\overset{k_b}{\rightleftharpoons}} {}^{Enz}Ab_{(x\text{-}IgE)} - IC$$

${}^{Enz}Ab_{(x\text{-}IgE)}$ = Enzyme labeled Antibody (Excess Quantity)
${}^{Enz}Ab_{(x\text{-}IgE)} - IC$ = Antigen-Antibodies Complex
k_b = Rate Constant of Association
k_{-b} = Rate Constant of Dissociation

4.0 REAGENTS

Materials Provided:

A. IgE Calibrators – 1.0 ml/vial - Icons A-F
Six (6) vials of human serum based reference calibrators at concentrations of 0 (**A**), 5 (**B**), 25 (**C**), 50 (**D**), 150 (**E**) and 400 (**F**) IU/ml. Store at 2-8°C. A preservative has been added.
Note: The Calibrators are standardized against WHO's 2ndIRP 75/502 for IgE

B. IgE Biotin Reagent – 13 ml/vial – Icon ▽
One (1) vial containing biotinylated anti-human IgE mIgG reagent presented in a protein-stabilized matrix. A preservative has been added. Store at 2-8°C.

C. IgE Enzyme Reagent – 13 ml/vial - Icon
One (1) vial containing anti-human IgE-HRP incorporated complex in a protein-stabilized matrix. A preservative has been added. Store at 2-8°C.

D. Streptavidin Plate – 96 wells – Icon ⇓
One 96-well microplate coated with streptavidin and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

E. Wash Solution Concentrate – 20ml/vial - Icon
One (1) vial containing a surfactant in buffered saline. A preservative has been added. Store at 2-8°C.

F. Substrate A – 7.0ml/vial - Icon S^A
One (1) vial containing tetramethylbenzidine (TMB) in acetate buffer. Store at 2-8°C.

G. Substrate B – 7.0ml/vial - Icon S^B
One (1) vial containing hydrogen peroxide (H_2O_2) in acetate buffer. Store at 2-8°C.

H. Stop Solution – 8.0ml/vial - Icon
One (1) vial containing a strong acid (1N HCl). Store at 2-8°C.

I. Product Instructions.

Note 1: Do not use reagents beyond the kit expiration date.
Note 2: Avoid extended exposure to heat and light. **Opened reagents are stable for sixty (60) days when stored at 2-8°C. Kit and component stability are identified on label.**
Note 3: Above reagents are for a single 96-well microplate.

4.1 Required But Not Provided:
1. Pipette capable of delivering 0.025 and 0.050ml (25 & 50µl) volumes with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100 and 0.350ml (100 & 350µl) volumes with a precision of better than 1.5%.
3. Microplate washers or a squeeze bottle (optional).
4. Microplate Reader with 450nm and 620nm wavelength absorbance capability.
5. Absorbent Paper for blotting the microplate wells.
6. Plastic wrap or microplate cover for incubation steps.
7. Vacuum aspirator (optional) for wash steps.
8. Timer.
9. Quality control materials.

5.0 PRECAUTIONS

For In Vitro Diagnostic Use
Not for Internal or External Use in Humans or Animals

All products that contain human serum have been found to be non-reactive for Hepatitis B Surface Antigen, HIV 1&2 and HCV Antibodies by FDA licensed reagents. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Edition, 1988, HHS Publication No. (CDC) 88-8395.

Safe disposal of kit components must be according to local regulatory and statutory requirement.

6.0 SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood serum in type and the usual precautions in the collection of venipuncture samples should be observed. For accurate comparison to established normal values, a fasting morning serum sample should be obtained. The blood should be collected in a plain redtop venipuncture tube without additives or anti-coagulants. Allow the blood to clot for samples. Centrifuge the specimen to separate the serum from the cells.

In patients receiving therapy with high biotin doses (i.e. >5mg/day), no sample should be taken until at least 8 hours after the last biotin administration, preferably overnight to ensure fasting sample.

Samples may be refrigerated at 2-8°C for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20°C for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.050ml (50µl) of the specimen is required.

7.0 QUALITY CONTROL

Each laboratory should assay controls at levels in the low, normal and elevated range for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations

8.0 REAGENT PREPARATION

1. **Wash Buffer**
Dilute contents of wash concentrate to 1000ml with distilled or deionized water in a suitable storage container. Storediluted buffer at 2-30°C for up to 60 days.
2. **Working Substrate Solution** – Stable for one year
Pour the contents of vial labeled Solution 'A' into the vial labeled Solution 'B'. Place the yellow cap on the mixed reagent for easy identification. Mix and label accordingly. Store at 2-8 °C.

Note 1: Do not use the working substrate if it looks blue.
Note 2: Do not use reagents that are contaminated or have bacteria growth.

9.0 TEST PROCEDURE

Before proceeding with the assay, bring all reagents, serum reference calibrators and controls to room temperature (20-27°C).
*****Test procedure should be performed by a skilled individual or trained professional*****

1. Format the microplates' wells for each serum reference calibrator, control and patient specimen to be assayed in duplicate. **Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.**
2. Pipette 0.025 ml (25µl) of the appropriate serum reference calibrator, control or specimen into the assigned well.
3. Add 0.100 ml (100µl) of the IgE Biotin Reagent to each well. **It is very important to dispense all reagents close to the bottom of the coated well.**
4. Swirl the microplate gently for 20-30 seconds to mix and cover.
5. Incubate 30 minutes at room temperature.
6. Discard the contents of the microplate by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper.
7. Add 0.350ml (350µl) of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.**
8. Add 0.100 ml (100µl) of the IgE Enzyme Reagent labeled antibody to each well.
DO NOT SHAKE THE PLATE AFTER ENZYME ADDITION
9. Cover and incubate 30 minutes at room temperature.
10. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.
11. Add 0.350ml (350µl) of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.**
12. Add 0.100 ml (100µl) of working substrate solution to all wells (see Reagent Preparation Section). **Always add reagents in the same order to minimize reaction time.**
DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION
13. Incubate at room temperature for fifteen (15) minutes.
14. Add 0.050ml (50µl) of stop solution to each well and gently mix for 15-20 seconds.
15. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) in a microplate reader. **The results should be read within thirty (30) minutes of adding the stop solution.**

10.0 CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of IgE in unknown specimens.
1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the absorbance for each duplicate serum reference versus the corresponding IgE concentration in IU/ml on linear graph

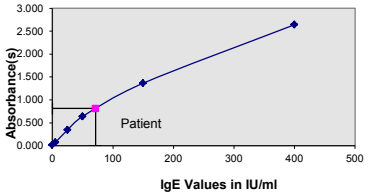
paper (do not average the duplicates of the serum references before plotting).
3. Draw the best-fit curve through the plotted points.
4. To determine the concentration of IgE for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in IU/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance (1.323) intersects the dose response curve at 142 IU/ml IgE concentration (See Figure 1).

Note: Computer data reduction software designed for ELISA assays may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained.

EXAMPLE 1

Sample I.D.	Well	Abs	Mean Abs (B)	Conc
Cal A	A1	0.014	0.015	0
	B1	0.016		
Cal B	C1	0.072	0.073	5
	D1	0.074		
Cal C	E1	0.364	0.345	25
	F1	0.326		
Cal D	G1	0.663	0.639	50
	H1	0.614		
Cal E	A2	1.340	1.364	150
	B2	1.388		
Cal F	C2	2.601	2.641	400
	D2	2.682		
Ctrl 1	E2	2.575	2.562	***375.3***
	F2	2.549		
Ctrl 2	G2	0.818	0.813	***71.2***
	H2	0.807		
Patient 1	A3	1.322	1.323	***142.0***
	B3	1.324		

Figure 1



*The data presented in Example 1 and Figure 1 is for illustration only and **should not** be used in lieu of a standard curve prepared with each assay.

11.0 Q.C. PARAMETERS

In order for the assay results to be considered valid the following criteria should be met:
1. The absorbance (OD) of calibrator 'A' should be ≤ 0.05
2. The absorbance (OD) of calibrator 'F' should be ≥ 1.3
3. Four out of six quality control pools should be within the established ranges.

12.0 RISK ANALYSIS

The MSDS and Risk Analysis Form for this product are available on request from Monobind Inc.

12.1 Assay Performance
1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. The addition of substrate solution initiates a kinetic reaction, terminated by the addition of the stop solution. Therefore, the substrate and stop solution should be added in the same sequence to eliminate any time-deviation during reaction.
6. Plate readers measure vertically. Do not touch the bottom of the wells.
7. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
8. Use components from the same lot. No intermixing of reagents from different batches.
9. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from Monobind IFU may yield inaccurate results.
10. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.
11. It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.
12. Risk Analysis- as required by CE Mark IVD Directive 98/79/EC - for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com.

12.2 Interpretation
1. ***Measurements and interpretation of results must be performed by a skilled individual or trained professional.***
2. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.
3. The reagents for the test system have been formulated to eliminate maximal interference; however, potential interaction between rare serum specimens and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for all kinds of immunoassays (Boscato LM, Stuart MC. 'Heterophilic antibodies: a problem for all immunoassays' Clin. Chem. 1988:3427-33). For diagnostic purposes, the results from this assay should be in combination with clinical examination, patient history and all other clinical findings.
4. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
5. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, Monobind shall have no liability.
6. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.
7. Serum IgE concentration is dependent upon a multiplicity of factors: including if the patient is sensitized, how many times the patient has been exposed to a specific allergen etc. Total IgE concentration alone is not sufficient to assess the clinical status. All the clinical findings especially specific allergy testing should be taken into consideration while determining the clinical status of the patient.
8. Since all atopic reactions are not IgE mediated, all relevant clinical information should be taken into consideration before making any determination for patients who may be in the normal range.

13.0 EXPECTED RANGES OF VALUES

A study of population from different age groups was conducted to evaluate the IgE AccuBind® ELISA test system. The results are presented in Table 1:

TABLE 1
Expected Values for the IgE (In IU/ml)

Age (Yrs)	Number (n)	Median	Absolute Range
0-3	31	6.4	ND - 46
3-16	43	25.0	ND – 280
Adult	145	43	0 - 200

It is important to keep in mind that establishment of a range of values which can be expected to be found by a given method for a population of "normal"-persons is dependent upon a multiplicity of factors: the specificity of the method, the population tested and the precision of the method in the hands of the analyst. For these reasons each laboratory should depend upon the range of expected values established by the Manufacturer only until an in-house range can be determined by the analysts using the method with a population indigenous to the area in which the laboratory is located.

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision
The within and between assay precision of the IgE AccuBind® ELISA Test System were determined by analyses on three different levels of pool control sera. The number, mean value, standard deviation and coefficient of variation for each of these control sera are presented in Table 2 and Table 3.

TABLE 2
Intra-Assay Precision (in IU/ml)

SAMPLE	N	X	σ	C.V.%
Low	20	48.9	2.87	5.87
Medium	20	160.5	6.47	4.03
High	20	297.6	5.81	1.95

TABLE 3
Inter Assay Precision (in IU/ml)

SAMPLE	N	X	σ	C.V.%
Low	10	46.3	3.9	8.42
Medium	10	157.0	7.3	4.64
High	10	301.0	10.6	3.52

14.2 Sensitivity
The IgE AccuBind® ELISA test system has a sensitivity of 0.125 IU/ml. The sensitivity was ascertained by determining the variability of the 0 IU/ml serum calibrator and using the 2σ (95% certainty) statistics to calculate the minimum dose.

14.3 Accuracy
The IgE AccuBind® ELISA test system was compared with a reference method. Biological specimens with IgE levels in the low, medium and high ranges were used. The values ranged from 0.8 to 3100 IIU/ml. The total number of such specimens was 219. The least square regression equation and the correlation coefficient were computed for this IgE AccuBind® ELISA method in comparison with the predicate method (Table 4):

TABLE 4

Method	Mean	Least Square Regression Analysis	Correlation Coefficient
Monobind (X)	179	x= -12.9 + 1.21(Y)	0.967
Predicate (Y)	157		

Only slight amounts of bias between this method and the reference method are indicated by the closeness of the mean values. The least square regression equation and correlation coefficient indicates excellent method agreement.

14.4 Specificity
The specificity of the IgE AccuBind® ELISA test system, to closely related immunoglobulins was evaluated by adding those at twice the physiological concentrations to a serum matrix. No cross-reaction between the antibodies used and the related molecules was detected.

14.5. High Dose Effect
Since the assay is sequential in design, high concentrations of IgE do not show the hook effect. Myeloma IgE patient samples with concentrations over 8 million IU/ml demonstrated extremely high levels of absorbance.

14.6 Linearity
Two patient pools were assayed diluted (in 'A' Calibrator) and undiluted with the IgE AccuBind® ELISA test system. The observed and expected values are listed below in Table 5:

TABLE 5

Sample	Observed (O) (IU/ml)	Expected (E) (IU/ml)	% Recovery (O/E)
Pool 1	106.8	-	-
Pool 1/2	50.8	53.4	95.1
Pool 1/4	25.3	26.7	94.8
Pool 1/8	13.4	13.3	100.6
Pool 1/16	6.6	6.7	98.5
Pool 2	395.9	-	-
Pool 2/2	189.5	197.9	95.8
Pool 2/4	106.1	98.9	107.2
Pool 2/8	48.0	49.5	96.9
Pool 2/16	25.8	24.7	104.2

14.7 Recovery
Two patient pools were spiked with known amounts of IgE and assayed with the IgE AccuBind® ELISA test system. The observed and expected values are listed below in Table 6.

TABLE 6

Sample	Observed (O) (IU/ml)	Expected (E) (IU/ml)	% Recovery (O/E)
Pool 1	25.7	-	-
Pool 1+ 25	50.7	50.7	100.0
Pool 1+ 50	74.8	75.7	101.2
Pool 1+ 100	122.7	125.7	97.6
Pool 1+ 200	232.0	225.7	102.7
Pool 2	12.3	-	-
Pool 2 + 25	41.7	37.3	111.2
Pool 2+ 50	62.6	62.3	100.6
Pool 2+ 100	109.4	112.3	97.4
Pool 2+ 200	197.2	212.3	92.8

15.0 REFERENCES

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Glossary of Symbols
(EN 960/ISO 15223)

IVD — In Vitro - Diagnostic Medical Device
Temperature Limitation Storage Condition (2-8° C)
Consult Instructions for Use
REF — Catalogue Number
Contains Sufficient Test for Σ
LOT — Batch Code
Used By (Expiration Day)
Date of Manufacturer
Manufacturer
EC REP — Authorized Rep in European Country
CE — European Conformity

ВЕКТОР БЕСТ

IgG общий-ИФА-БЕСТ

A-8662

Набор реагентов
для иммуноферментного определения
концентрации общего иммуноглобулина
класса G в сыворотке крови

ИНСТРУКЦИЯ ПО ПРИМЕНЕНИЮ

Утверждена 14.08.2018

1. НАЗНАЧЕНИЕ

1.1. Набор реагентов для иммуноферментного определения концентрации общего иммуноглобулина класса G в сыворотке крови «IgG общий-ИФА-БЕСТ» (далее по тексту – набор) предназначен для определения концентрации общего иммуноглобулина класса G ($IgG_{общ}$) в сыворотке крови человека методом твердофазного иммуноферментного анализа.

1.2. Набор рассчитан на проведение анализа в дублях 41 неизвестного, 6 калибровочных и 1 контрольного образцов (всего 96 определений при использовании всех стрипов планшета).

2. ХАРАКТЕРИСТИКА НАБОРА

2.1. Принцип метода

Метод определения основан на двухстадийном «сэндвич»-варианте твердофазного иммуно-ферментного анализа с применением моноклональных антител к IgG.

На первой стадии калибровочные образцы с известной концентрацией $IgG_{общ}$ и анализируемые образцы инкубируются в лунках стрипированного планшета с иммобилизованными моноклональными антителами (МКАТ) к гамма-цепям IgG. На второй стадии связавшийся в лунках IgG обрабатывают конъюгатом МКАТ к легким (лямбда и каппа) цепям иммуноглобулинов человека с пероксидазой.

Образовавшиеся иммунные комплексы «иммобилизованные МКАТ – IgG – конъюгат» выявляют ферментативной реакцией с раствором тетраметилбензидина. Степень окрашивания пропорциональна концентрации $IgG_{общ}$ в анализируемом образце. После измерения величины оптической плотности раствора в лунках на основании калибровочного графика рассчитывается концентрация $IgG_{общ}$ в анализируемых образцах.

2.2. Состав набора

В состав набора входят:

- планшет разборный (12 восьмилуночных стрипов) с иммобилизованными на внутренней поверхности лунок моноклональными антителами к гамма-цепям IgG человека, готовый для использования – 1 шт.;
- калибровочные образцы, содержащие известные количества $IgG_{общ}$ – 0; 17,5; 35; 75; 150; 300 Ед/мл (0; 1,4; 2,8; 6; 12 и 24 мг/мл), аттестованные относительно WHO International Standard Immunoglobulins G, A and M, human serum, NIBSC 67/086; концентрации IgGобщ в калибровочных образцах могут несколько отличаться от указанных величин, точные величины указаны на этикетках флаконов, готовые для использования – 6 флаконов (по 0,5 мл);
- контрольный образец на основе инактивированной сыворотки крови человека с известным содержанием $IgG_{общ}$, аттестованный относительно WHO International Standard Immunoglobulins G, A and

M, human serum, NIBSC 67/086; готовый для использования – 1 флакон (0,5 мл);
– конъюгат моноклональных антител к легким (лямбда и каппа) цепям иммуноглобулинов человека с пероксидазой хрена, готовый для использования – 1 флакон (13 мл);
– раствор для разведения сывороток (РРС), концентрат – 1 флакон (28 мл);
– концентрат фосфатно-солевого буферного раствора с твином (ФСБ-Т×25) – 1 флакон (28 мл);
– раствор тетраметилбензидина плюс (раствор ТМБ плюс), готовый для использования – 1 флакон (13 мл);
– стоп-реагент, готовый для использования – 1 флакон (12 мл);
– пленка для заклеивания планшета – 2 шт.;
– ванночка для реагентов – 2 шт.;
– наконечники для пипеток на 5–200 мкл – 16 шт.;
– планшет для предварительного разведения исследуемых образцов – 1 шт.

Принадлежности:
– трафарет для построения калибровочного графика – 1 шт.

3. АНАЛИТИЧЕСКИЕ ХАРАКТЕРИСТИКИ

3.1. Специфичность. В наборе «IgG общий – ИФА – БЕСТ» используются моноклональные антитела, обладающие высокой специфичностью к гамма-цепям IgG. Перекрестного связывания с IgM, IgA, IgE или альбумином в физиологических концентрациях не наблюдалось.

3.2. «Хук»-эффект при использовании набора реагентов не зафиксирован. Оптическая плотность образцов сыворотки крови с концентрацией $IgG_{общ}$ до 1000 Ед/мл всегда превышала оптическую плотность калибровочного образца с максимальной концентрацией $IgG_{общ}$.

3.3. *Воспроизводимость. Коэффициент вариации результатов определения концентрации $IgG_{общ}$ в лунках, содержащих контрольный образец, не превышает 8%.

3.4. *Линейность. Данный аналитический параметр проверяется тестом на «линейность» – отклонение от расчетной величины концентрации $IgG_{общ}$ при разведении калибровочных образцов, содержащих 300, 150, 75, 35 Ед/мл в 2 раза. Процент «линейности» составляет: 90–110 %.

3.5. *Точность. Данный аналитический параметр проверяется тестом на «открытие» – соответствие измеренной концентрации $IgG_{общ}$ расчетному значению в пробе, полученной путем смешивания равных объемов контрольного образца и калибровочного образца с концентрацией $IgG_{общ}$ 35 Ед/мл. Процент «открытия» составляет 90–110%.

3.6. *Чувствительность. Минимально определяемая концентрация $IgG_{общ}$, рассчитанная на основании среднего арифметического значения оптической плотности калибровочного образца B_0 (с концентрацией $IgG_{общ}$ 0 Ед/мл) плюс 2σ (σ - среднее квадратичное отклонение

от среднего арифметического значения B_0) не превышает 2,5 Ед/мл (0,2 мг/мл).

3.7. Клиническая проверка. Концентрация $IgG_{общ}$, измеренная в сыворотке крови условно здоровых доноров находилась в диапазоне 37–200 Ед/мл (см. стр. 29).

3.8. Рекомендуется в каждой лаборатории при использовании набора уточнить значения концентрации $IgG_{общ}$, соответствующие нормальным для данного региона у обследуемого контингента людей.

4. МЕРЫ ПРЕДОСТОРОЖНОСТИ

4.1. Потенциальный риск применения набора – класс 2а (Приказ МЗ РФ от 06.06.2012 № 4н).

4.2. Все компоненты набора являются нетоксичными. Стоп-реагент обладает раздражающим действием. Избегать разбрызгивания и попадания на кожу и слизистые. В случае попадания стоп-реагента на кожу и слизистые необходимо промыть пораженный участок большим количеством проточной воды.

4.3. При работе с исследуемыми образцами следует соблюдать меры предосторожности, принятые при работе с потенциально инфекционным материалом. Основные правила работы изложены в «Инструкции по мерам профилактики распространения инфекционных заболеваний при работе в клинико-диагностических лабораториях лечебно-профилактических уч-

реждений», утвержденной Минздравом СССР 17 января 1991 г. и в методических указаниях МУ 287-113 «Методические указания по дезинфекции, предстерилизационной очистке и стерилизации изделий медицинского назначения», утв. департаментом госсанэпиднадзора Минздрава РФ от 30.12.1998.

4.4. При работе с набором следует надевать одноразовые резиновые или пластиковые перчатки, так как образцы сыворотки крови человека следует рассматривать как потенциально инфекционные, способные длительное время сохранять и передавать ВИЧ, вирусы гепатита или возбудителей других инфекций.

4.5. Лабораторная посуда и оборудование, которые используются в работе с набором, должны быть соответствующим образом промаркированы и храниться отдельно.

4.6. Запрещается прием пищи, использование косметических средств и курение в помещениях, предназначенных для работы с наборами.

4.7. Для дезинфекции посуды и материалов, контактировавших с исследуемыми и контрольными образцами, рекомендуем использовать дезинфицирующие средства, не оказывающие негативного воздействия на качество ИФА, не содержащие активный кислород и хлор, например, комбинированные средства на основе ЧАС,

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* по ГОСТ Р 51352-2013.

спиртов, третичных аминов. Использование дезинфицирующих средств, содержащих активный кислород и хлор (H_2O_2, деохлор, хлорамин), приводит к серьезному искажению результатов.

4.8. При использовании набора образуются отходы классов А, Б и Г, которые классифицируются и уничтожаются (утилизируются) в соответствии с СанПиН 2.1.7.2790-10 «Санитарно-эпидемиологические требования к обращению с медицинскими отходами». Дезинфекцию наборов реагентов следует проводить по МУ 287-113 «Методические указания по дезинфекции, предстерилизационной очистке и стерилизации изделий медицинского назначения».

5. ОБОРУДОВАНИЕ И МАТЕРИАЛЫ, НЕОБХОДИМЫЕ ДЛЯ РАБОТЫ С НАБОРОМ:

- Спектрофотометр вертикального сканирования, позволяющий проводить измерения оптической плотности растворов в лунках стрипов при основной длине волны 450 нм и длине волны сравнения в диапазоне 620 – 655 нм; допускается измерение только при длине волны 450 нм;
- шейкер термостатируемый орбитального типа, позволяющий производить встряхивание при температуре 37±1°С и 400–800 об/мин;
- микроцентрифуга, позволяющая центрифугировать при 1500–2000 об/мин;
- промывочное устройство для планшетов;
- холодильник бытовой;

– пипетки полуавтоматические одноканальные с переменным или фиксированным объемом со сменными наконечниками, позволяющие отбирать объемы жидкости от 5 до 5000 мкл;
– пипетка полуавтоматическая многоканальная со сменными наконечниками, позволяющая отбирать объемы жидкостей от 5 до 350 мкл;
– флаконы стеклянные вместимостью 15 мл;
– цилиндр мерный вместимостью 1000 мл;
– вода дистиллированная;
– перчатки медицинские диагностические одноразовые;
– бумага фильтровальная лабораторная;
– дезинфицирующий раствор.

6. АНАЛИЗИРУЕМЫЕ ОБРАЗЦЫ

6.1. Для проведения анализа не следует использовать гемолизованную, мутную сыворотку крови.

6.2. Образцы сыворотки крови можно хранить при температуре от 2 до 8°С не более 48 часов, при температуре минус 20°С (и ниже) не более 3 месяцев. Повторное замораживание и размораживание образцов сыворотки крови не допускается. После размораживания образцы следует тщательно перемешать.

6.3. Образцы сывороток крови, содержащие осадок, необходимо очистить центрифугированием при 1500 об/мин в течение 5 мин при температуре от 18 до 25°С.

7. ПРОВЕДЕНИЕ АНАЛИЗА

ПОДГОТОВКА РЕАГЕНТОВ

7.1. Перед проведением анализа компоненты набора и исследуемые образцы следует выдержать при температуре от 18 до 25°С не менее 30 мин.

7.2. Подготовка планшета

Вскрыть пакет выше замка и установить на рамку необходимое для проведения анализа количество стрипов. Использовать в течение 1 часа после установки. Оставшиеся стрипы немедленно поместить вновь в пакет с влагопоглотителем, удалить из него воздух, плотно закрыть замок.

Хранить при температуре от 2 до 8°С в течение всего срока годности набора.

7.3. Приготовление промывочного раствора

Раствор готовится из концентрата фосфатно-солевого буферного раствора. При выпадении осадка солей в концентрате необходимо прогреть его при температуре 30–40°С до полного растворения осадка.

Внести в мерный цилиндр необходимое количество концентрата фосфатно-солевого буферного раствора с твином (ФСБ-Т×25) и добавить соответствующее количество дистиллированной воды.

В таблице приведен расход реагента в зависимости от количества используемых стрипов.

Приготовленный промывочный раствор можно хранить при температуре от 2 до 8°С не более 5 сут.

7.4. Приготовление рабочего раствора для разведения сывороток

Приготовить за 30 мин до начала постановки анализа.

При выпадении осадка солей в концентрате РРС необходимо прогреть его при температуре 30–40°С до полного растворения осадка.

Внести в мерный цилиндр необходимое количество концентрата раствора для разведения сывороток и добавить соответствующее количество дистиллированной воды.

В таблице приведен расход реагента в зависимости от количества используемых стрипов.

Приготовленный рабочий раствор для разведения сывороток можно хранить при температуре от 2 до 8°С не более 3 сут.

7.5. Подготовка калибровочных образцов и контрольного образца

Калибровочные образцы и контрольный образец готовы к использованию и не требуют дополнительного разведения. Перед использованием флаконы встряхнуть или центрифугировать на микроцентрифуге так, чтобы капли

растворов со стенок и крышки опустились на дно. Затем содержимое флаконов тщательно перемешать на вортексе или пипетированием, избегая образования пены.

Калибровочные образцы и контрольный образец после вскрытия можно хранить в плотно закрытых флаконах при температуре от 2 до 8°С в течение всего срока годности набора.

7.6. Приготовление рабочего разведения анализируемых образцов сыворотки крови

Готовится в стеклянных заранее промаркированных флаконах за 5–10 мин до начала постановки анализа.

В чистый флакон с 10 мл рабочего раствора для разведения сывороток (см. п. 7.4) добавить 10 мкл исследуемой сыворотки и тщательно перемешать. Таким образом, рабочее разведение сыворотки составляет 1000 раз*.

Использовать в течение 30 мин после приготовления.

7.7. Подготовка конъюгата.

Конъюгат готов к использованию.

Необходимое количество конъюгата отобрать в чистый флакон или ванночку для реагента.

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* См. также раздел «Дополнительная информация для потребителей», п. 2

Оставшийся после проведения ИФА конъюгат утилизировать ***(не сливать во флакон с исходным конъюгатом).***

В таблице приведен расход реагента в зависимости от количества используемых стрипов.

Конъюгат после вскрытия можно хранить в плотно закрытом флаконе при температуре от 2 до 8°С в течение всего срока годности набора.

7.8. Подготовка раствора тетраметилбензидина плюс.

Раствор ТМБ плюс готов к использованию.

Необходимое количество раствора ТМБ плюс отобрать в чистый флакон или ванночку для реагента.

Оставшийся после проведения ИФА раствор ТМБ плюс утилизировать ***(не сливать во флакон с исходным раствором ТМБ плюс).***

Необходимо исключить воздействие прямого света на раствор ТМБ плюс.

Раствор ТМБ плюс после вскрытия можно хранить в плотно закрытом флаконе при температуре от 2 до 8°С в течение всего срока годности набора.

В таблице приведен расход реагента в зависимости от количества используемых стрипов.

7.9. Стоп-реагент готов к использованию.

После первого вскрытия стоп-реагент можно хранить в плотно закрытом флаконе

Таблица 1

Кол-во используемых стрипов	Промывочный раствор		Рабочий раствор для разведения сывороток		Конъюгат, мл	Раствор ТМБ плюс, мл
	ФСБ-Т×25, концентрат, мл	Дистил. вода, мл	РРС, концентрат, мл	Дистил. вода, мл		
2	4,0	до 100	4,0	до 100	2,0	2,0
3	6,0	до 150	6,0	до 150	3,0	3,0
4	8,0	до 200	8,0	до 200	4,0	4,0
5	10,0	до 250	10,0	до 250	5,0	5,0
6	12,0	до 300	12,0	до 300	6,0	6,0
7	14,0	до 350	14,0	до 350	7,0	7,0
8	16,0	до 400	16,0	до 400	8,0	8,0
9	18,0	до 450	18,0	до 450	9,0	9,0
10	20,0	до 500	20,0	до 500	10,0	10,0
11	22,0	до 550	22,0	до 550	11,0	11,0
12	24,0	до 600	24,0	до 600	12,0	12,0

при температуре от 2 до 8°С в течение всего срока годности набора.

ПРОВЕДЕНИЕ ИФА

7.10. Внести во все лунки по 100 мкл рабочего раствора для разведения сывороток (см п. 7.4).

Внести в соответствующие лунки в дублях, начиная с верхних лунок первых двух стрипов, по 20 мкл каждого калибровочного образца. В следующую пару лунок внести по 20 мкл контрольного образца. В остальные лунки внести

в дублях по 20 мкл анализируемых образцов сыворотки крови в рабочем разведении (см п. 7.6), каждый раз меняя наконечник.

Время внесения образцов не должно превышать 10 мин при использовании всех лунок планшета.

7.11. Планшет заклеить пленкой и инкубировать в течение 20 мин при встряхивании на термостатируемом шейкере при температуре 37±1°С и 700 об/мин.

7.12. По окончании инкубации снять липкую пленку и удалить ее в сосуд с дезинфицирующим раствором. Содержимое лунок удалить отсасыванием в сосуд с дезинфицирующим раствором и промыть, добавляя во все лунки по 350 мкл промывочного раствора. Процесс промывки повторить еще 4 раза. Общее количество отмывок равно 5. Время между заполнением и опорожнением лунок должно быть не менее 30 сек. Необходимо следить за полным опорожнением лунок после каждого цикла отмывки. Затем удалить остатки жидкости из лунок, постукивая планшетом в перевернутом положении по фильтровальной бумаге.

7.13. Внести во все лунки планшета по 100 мкл конъюгата (см п. 7.7).

Для внесения конъюгата использовать ванночку для реагента и одноразовые наконечники, входящие в состав набора.

7.14. Планшет заклеить пленкой и инкубировать в течение 20 мин при встряхивании на

термостатируемом шейкере при температуре 37±1°С и 700 об/мин.

7.15. По окончании инкубации удалить содержимое лунок и промыть планшет, как это указано в п. 7.12.

7.16. Внести во все лунки по 100 мкл раствора ТМБ плюс (см п. 7.8) и инкубировать в защищенном от света месте в течение 15 мин при температуре от 18 до 25°С.

Для внесения раствора ТМБ плюс использовать ванночку для реагента и одноразовые наконечники, входящие в состав набора.

7.17. Внести во все лунки с той же скоростью и в той же последовательности, как и раствор ТМБ плюс, по 100 мкл стоп-реагента, при этом содержимое лунок окрашивается в желтый цвет.

8. РЕГИСТРАЦИЯ РЕЗУЛЬТАТОВ

Измерить величину оптической плотности растворов в лунках стрипов на спектрофотометре вертикального сканирования в двухволновом режиме: основной фильтр – 450 нм, референс-фильтр в диапазоне 620–655 нм; допускается измерение только с фильтром 450 нм. Измерение проводить через 2–3 мин после остановки реакции.

Время между остановкой реакции и измерением оптической плотности не должно превышать 10 мин.

9. УЧЕТ РЕЗУЛЬТАТОВ

9.1. Вычислить средние арифметические значения оптической плотности для каждой пары лунок, содержащих калибровочные образцы.

9.2. Построить в линейных координатах калибровочный график зависимости среднего арифметического значения оптической плотности (ед. опт. плотн.) от концентрации $IgG_{общ}$ в калибровочных образцах (Ед/мл или мг/мл).

9.3. Определить концентрацию $IgG_{общ}$ в контрольном образце и анализируемых образцах по калибровочному графику. Вычислить среднее арифметическое значение концентрации для каждой пары лунок, содержащих анализируемые образцы.

9.4. Если при проведении анализа использовали разведение сыворотки в 1000 раз (базовое разведение для данного набора), то найденное по графику количество $IgG_{общ}$ соответствует концентрации $IgG_{общ}$ в анализируемом образце в Ед/мл (мг/мл). Если использовали другое разведение образца, то найденное по графику количество $IgG_{общ}$ пересчитывают с учетом дополнительного разведения, также получая в результате концентрацию $IgG_{общ}$ в Ед/мл (мг/мл).

Если значение оптической плотности анализируемого образца превышает значение ОП для калибровочного образца 300 Ед/мл (24 мг/мл), то данный образец анализируют повторно после дополнительного разведения в 2 раза, полученный результат умножают на 2.

10. УСЛОВИЯ ТРАНСПОРТИРОВАНИЯ, ХРАНЕНИЯ И ПРИМЕНЕНИЯ НАБОРА

10.1. Транспортировать изделия следует транспортом всех видов в крытых транспортных средствах в соответствии с правилами перевозок, действующими на транспорте данного вида, при температуре от 2 до 8°С. Допускается транспортирование при температуре до 25°С не более 10 суток.

10.2. Хранение набора в упаковке предприятия-изготовителя должно осуществляться при температуре от 2 до 8°С в течение всего срока годности в холодильных камерах или холодильниках, обеспечивающих регламентированный температурный режим с ежедневной регистрацией температуры.

10.3. Срок годности набора – 12 месяцев со дня выпуска. Не допускается применение наборов по истечении срока их годности.

10.4. Дробное использование набора может быть реализовано в течение всего срока годности.

В случае дробного использования набора:

– неиспользованные стрипы можно хранить в плотно закрытом пакете при температуре от 2 до 8°С в течение всего срока годности набора;
– калибровочные образцы, контрольный образец и конъюгат после вскрытия можно хранить в плотно закрытых флаконах при температуре от 2 до 8°С в течение всего срока годности набора;
– концентрат фосфатно-солевого буферного раствора с твином, концентрат раствора для разведения

сывороток; раствор ТМБ плюс и стоп-реагент после вскрытия можно хранить в плотно закрытых флаконах при температуре от 2 до 8°С в течение всего срока годности набора;

– рабочий раствор для разведения сывороток можно хранить при температуре от 2 до 8°С не более 3 сут;
– промывочный раствор можно хранить при температуре от 2 до 8°С не более 5 сут.

10.5. Построение калибровочного графика необходимо проводить для каждого независимого эксперимента, рекомендуется также каждый раз определять концентрацию $IgG_{общ}$ в контрольном образце.

10.6. Для перевода результатов измерений концентрации общего иммуноглобулина класса G из Ед/мл в мг/мл следует использовать коэффициент пересчета 0,08 (1 Ед/мл $IgG_{общ}$ = 0,08 мг/мл $IgG_{общ}$).

10.7. При постановке ИФА нельзя использовать компоненты из наборов разных серий или смешивать их при приготовлении растворов, кроме неспецифических компонентов (ФСБ-Т×25, раствор ТМБ плюс, стоп-реагент), которые взаимозаменяемы во всех наборах АО «Вектор-Бест».

10.8. Для получения надежных результатов необходимо строгое соблюдение инструкции по применению набора.

11. ГАРАНТИЙНЫЕ ОБЯЗАТЕЛЬСТВА

11.1. Производитель гарантирует соответствие выпускаемых изделий требованиям нормативной и технической документации.

Безопасность и качество изделия гарантируются в течение всего срока годности.

11.2. Производитель отвечает за недостатки изделия, за исключением дефектов, возникших вследствие нарушения правил пользования, условий транспортирования и хранения, либо действия третьих лиц, либо непреодолимой силы.

11.3. Производитель обязуется за свой счет заменить изделие, технические и функциональные характеристики (потребительские свойства) которого не соответствуют нормативной и технической документации, если указанные недостатки явились следствием скрытого дефекта материалов или некачественного изготовления изделия производителем.

По вопросам, касающимся качества набора «IgG общий-ИФА-БЕСТ», следует обращаться в АО «Вектор-БЕСТ» по адресу:
630559, Новосибирская область,
Новосибирский р-н,
р.п. Кольцово, а/я 121,
тел. (383) 363-20-60, 227-75-43,
тел./факс (383) 363-35-55.
E-mail: vbobtk@vector-best.ru

ДОПОЛНИТЕЛЬНАЯ ИНФОРМАЦИЯ ДЛЯ ПОТРЕБИТЕЛЕЙ

Набор предназначен для профессионального применения в клинической лабораторной диагностике обученным персоналом.

Требования безопасности к медицинским лабораториям приведены в ГОСТ Р 52905-2007.

Все реагенты наборов, содержащие в своем составе материалы человеческого происхождения, инактивированы.

При динамическом наблюдении пациента для получения результатов, адекватно отражающих изменение концентрации $IgG_{общ}$ в крови, необходимо использовать наборы реагентов одного наименования (одного предприятия-изготовителя).

1. Обеспечение получения правильных результатов анализа

Достоверность и воспроизводимость результатов анализа зависят от выполнения следующих основных правил:

– не проводите ИФА в присутствии паров кислот, щелочей, альдегидов или пыли, которые могут влиять на ферментативную активность конъюгатов;
– ферментативная реакция чувствительна к присутствию ионов металлов, поэтому не допускайте контактов каких-либо металлических предметов с конъюгатом и раствором ТМБ;

– избегайте загрязнения компонентов набора микроорганизмами и химическими примесями, для этого используйте в работе чистую посуду и чистые одноразовые наконечники для каждого реагента, контроля, образца;
– рабочие поверхности столов, оборудования обрабатывайте 70% этиловым спиртом (не допускается использование перекиси водорода, хлорсодержащих растворов);
– никогда не используйте одну и ту же емкость для конъюгата и раствора ТМБ;
– перед отбором ТМБ из флакона необходимо обрабатывать конус пипетки (внутреннюю и внешнюю поверхности) сначала дистиллированной водой, а затем 70% этиловым спиртом, так как малейшее загрязнение пипеток конъюгатом может привести к контаминации всего содержимого флакона с ТМБ;
– если допущена ошибка при внесении анализируемого образца, нельзя, опорожнив эту лунку, вносить в нее новый образец; такая лунка бракуется.

Качество промывки лунок планшета играет важную роль для получения правильных результатов анализа:

– Для аспирации анализируемых образцов и последующей промывки рекомендуется использовать автоматическое или ручное промывочное устройство.

– Не допускайте высыхания лунок планшета в перерыве между завершением промывки и внесением реагентов.
– Добивайтесь полного заполнения и опорожнения всех лунок планшета в процессе промывки. Недостаточная аспирация жидкости в процессе промывки может привести к понижению чувствительности и специфичности анализа.
– Следите за состоянием промывочного устройства – регулярно (1 раз в неделю) обрабатывайте шланги и емкости 70% этиловым спиртом.
– Для предотвращения засорения игл промывочного устройства в конце рабочего дня обязательно выполните процедуру ополаскивания системы подачи жидкости дистиллированной водой.

2. Рекомендации по подготовке анализируемых образцов

Вместо одноступенчатого (п. 7.6.) допустимо проводить двухступенчатое разведение сывороток с использованием планшета для предварительного разведения исследуемых образцов. Для этого, в каждую лунку планшета для предварительного разведения внести по 310 мкл рабочего раствора для разведения сывороток. Далее в одну из лунок, например, А-1, добавить 10 мкл исследуемой сыворотки, сменить исполь-

зованный наконечник пипетки на новый и затем с его помощью тщательно перемешать содержимое лунки (5–6 круговых движений, во время которых следует 3–4 раза набрать и опорожнить наконечник), избегая образования пены. После этого из лунки отобрать 10 мкл, внести в соседнюю лунку, например, А-2, и таким же образом тщательно перемешать (для этой операции также желательно использовать новый чистый наконечник пипетки). В лунке А-2 получаем рабочее разведение сыворотки 1000 раз. Аналогично развести и другие исследуемые сыворотки (например, в лунках В-1 и В-2, С-1 и т.д.):

– 310 мкл рабочего раствора для разведения сывороток + 10 мкл исследуемого образца → предварительное разведение образца в 32 раза;

– 310 мкл рабочего раствора для разведения сывороток + 10 мкл образца после предварительного разведения → рабочее разведение образца в 1000 раз.

Внимание! *Точность приготовления разведений определяет качество постановки теста!*

При исследовании не сыворотки, а других биологических жидкостей, степень разведения исследуемых образцов следует заранее подобрать опытным путем, используя как ориентир данные таблицы 2.

3. Условия правильности работы набора

Результаты анализа исследуемых образцов

Таблица 2

Абсолютные значения уровней содержания иммуноглобулинов в различных биологических жидкостях у здоровых лиц (M±δ)

(Тотолян А.А. Современные подходы к диагностике иммунопатологических состояний. Мед. иммунология, 1999 ,т.1 №1-2, с. 75-108)

Биологические жидкости	Содержание иммуноглобулинов классов:				
	***IgA*, г/л**	***IgM*, г/л**	***IgG*, г/л**	***sIgA*, г/л**	***IgE*, кЕ/л**
ЦСЖ	0,006±0,0013	0,0049±0,001	0,037±0,004	н/опр	0±0
Слюна	0,069±0,028	0,055±0,011	0,042±0,017	0,768±0,275	н/опр
Назальный смыв	0,014±0,006	0,025±0,017	0,042±0,017	0,071±0,022	0±0
Ларингеальный секрет	0,071±0,022	0,063±0,044	0,085±0,044	1,31±1,87	н/опр
Слезная жидкость	0,165±0,02	0,038±0,008	0,185±0,06	н/опр	н/опр
Эякулят	1,01±0,67	0,9±0,46	0,51±0,2	2,21±1,01	0±0
Сыворотка крови	2,15±0,85	1,63±0,46	12,3±2,97	0,79±0,22	50,0±12,5

Примечание: н/опр – данный показатель не определяли.

Приведенные показатели можно использовать только как ориентировочные, и в каждой лаборатории рекомендуется вычислить собственные границы нормальных значений концентрации общего IgG в сыворотке крови.

учитывать, если будут выполнены следующие условия:

– соотношение оптических плотностей калибровочных образцов: $ОП_0 < ОП_{17,5} < ОП_{35} < ОП_{75} < ОП_{150} < ОП_{300}$;

– $ОП_{300} \geq 1,0$ ед. опт. плотн. (о.е.);

– вычисленное по калибровочному графику значение концентрации $IgG_{общ}$ в контрольном образце попадает в пределы, указанные на этикетке флакона.

$ОП_0$, $ОП_{17,5}$, $ОП_{35}$, $ОП_{75}$, $ОП_{150}$ и $ОП_{300}$ – среднее значение оптической плотности калибровочных образов, содержащих 0; 17,5; 35; 75; 150 и 300 Ед/мл $IgG_{общ}$ соответственно.

4. Расчет результатов анализа

По результатам измерения вычислить среднее арифметическое значение оптической плотности (ОП) в лунках с анализируемыми образцами.

Построить в линейных координатах калибровочный график зависимости оптической плотности (ось ординат) от концентрации $IgG_{общ}$ (ось абсцисс) в калибровочных образцах. Для этого на прилагаемом трафарете для построения графика против концентрации каждого калибровочного образца отложить соответствующее ей среднее значение оптической плотности. Последовательно соединить полученные точки отрезками прямых линий.

Пример калибровочного графика представлен на рисунке.

Определить содержание $IgG_{общ}$ в контрольном и в анализируемых образцах по калибровочному графику. Для этого на оси ординат отметить значение ОП анализируемого образца. Провести прямую линию параллельно оси абсцисс до пересечения с калибровочным графиком. От точки пересечения опустить перпендикуляр на ось абсцисс. По полученной точке пересечения определить значение концентрации $IgG_{общ}$ в образце.

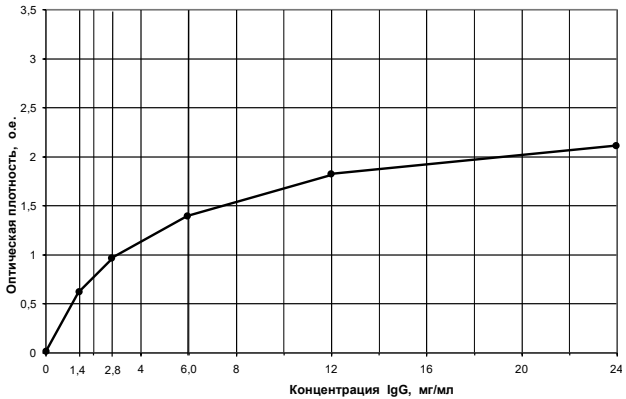

Рисунок. Зависимость оптической плотности от концентрации IgG в калибровочных пробах.

При использовании для расчетов концентраций компьютерного или встроенного в спектрофотометр программного обеспечения в настройках выбрать метод, соответствующий кусочно-линейной аппроксимации.

5. Диагностическая значимость

IgG, как и другие иммуноглобулины, относится к гуморальным факторам иммунитета. Карта гуморального иммунитета довольно индивидуальна, тем не менее пределы нормальных физиологических концентраций достаточно хорошо очерчены.

Диапазоны концентрации IgG в сыворотке крови здоровых доноров, начиная с 12 лет, составляют 5,3–16,5 мг/мл (Тотолян А.А., Марфичева Н.А., Тотолян Н.А. «Иммуноглобулины в клинической лабораторной диагностике», С-Пб, 1996).

По нашим собственным данным, концентрация $IgG_{общ.}$ в сыворотке крови условно здоровых мужчин и женщин из юго-восточного региона Западной Сибири в возрасте 20–50 лет (n=107) в основном находится в диапазоне 60–200 Ед/мл (4,8–16,0 мг/мл); а у детей из этого же региона в возрасте 1–12 лет – соответственно в диапазоне 37–194 Ед/мл (2,96–15,52 мг/мл). Следует, однако, учитывать, что нормальные значения концентраций $IgG_{общ.}$ могут довольно существенно отличаться в зависимости от реги-

она, возраста, экологических и многих других причин. Известно также, что для использования в диагностике часто бывает важнее знать не абсолютное значение концентрации общего иммуноглобулина, а его относительное отклонение от нормального местного, возрастного или, например, профессионального уровня. Поэтому нормальные региональные уровни общих иммуноглобулинов должны определяться каждой лабораторией самостоятельно.

У новорожденных детей уровень концентрации IgG в сыворотке крови часто такой же как у взрослых, однако уже через 1–2 месяца он уменьшается до 30–40% от исходного. Затем он медленно увеличивается, достигая к 6 месяцам 45% (в среднем), к 8–10 месяцам – 62%, к 6 годам – 90%, и только в 9–12 лет он, наконец, становится равным уровню взрослого человека. Отклонение концентрации IgG от нормы отражает состояние иммунной системы и может свидетельствовать о серьезном заболевании.

Результаты определения общего сывороточного иммуноглобулина G могут быть с успехом использованы для дифференциальной диагностики целого ряда заболеваний (см. Иммунограмму).

Во всех случаях более полную картину способно дать параллельное определение трех основных классов иммуноглобулинов – G, M и A, а также иммуноглобулина E (см. иммунограмму).

Иммунограмма при некоторых заболеваниях

	IgG	IgA	IgM	IgE
Заболевания печени				
Острый инфекционный гепатит	+	N/+	N/++	N
Хронический персистирующий гепатит	N/+	N	N/+	N/+
Хронический агрессивный гепатит	++	+	N/++	N/+
Постгепатитный криптогенный цирроз	++	+	+	N/+
Первичный билиарный цирроз	N/+	N	+/++	N
Алкогольный цирроз	N/+	++	N/+	N
Болезни почек				
Острый пиелонефрит	N	N	+/++	N
Хронический пиелонефрит	+/++	N	+/++	N/+
Нефротический синдром	—	—	N/—	N/—
Инфекционные заболевания				
Острая инфекция	N	N	+/++	N
Хроническая инфекция	+/++	N/+	N/+	N/+
Системные ревматические заболевания				
Ревматоидный артрит	N/++	N/++	N/+	+/++
Системная красная волчанка	+	N	N/+	N/+
Склеродермия	N	N	N	N/+
Смешанные системные заболевания	N/+	N/+	N	N/+
Атопия, аллергические заболевания	N/+	N	N/—	+/++
Гельминтозы и др. паразитарные заболевания	N/+	N/+	N/+	+/++

N – нормальная регионально-возрастная концентрация иммуноглобулина (в пределах нормального диапазона от Nmin до Nmax)

\+ – повышенная концентрация иммуноглобулина (от Nmax до 1,3Nmax)

++ – сильно повышенная концентрация иммуноглобулина (более 1,3Nmax)

– – пониженная концентрация иммуноглобулина (ниже Nmin)

6. Краткая схема проведения ИФА для набора реагентов «IgG общий-ИФА-БЕСТ»

Использовать только после внимательного ознакомления с инструкцией!

Внести:	по 100 мкл рабочего раствора для разведения сывороток;
	по 20 мкл калибровочных и контрольного образцов в дублях в контрольные лунки;
	по 20 мкл разведенных анализируемых образцов в дублях в лунки для исследуемых образцов.
Инкубировать:	20 мин, 37°С, 700 об/мин.
Промыть:	промывочный раствор, 350 мкл, 5 раз.
Внести:	по 100 мкл конъюгата.
Инкубировать:	20 мин, 37°С, 700 об/мин.
Промыть:	промывочный раствор, 350 мкл, 5 раз.
Внести:	по 100 мкл раствора ТМБ плюс.
Инкубировать:	15 мин, 18–25°С, в темноте.
Внести:	по 100 мкл стоп-реагента.
Измерить:	ОП при 450 нм / референсная длина волны 620–655 нм.

7. Графические символы

REF	Номер по каталогу	IVD	Медицинское изделие для диагностики *in vitro*
Σ n	Содержимого достаточно для проведения n-количества тестов	NON STERILE	Не стерильно
LOT	Код партии		Температурный диапазон
	Изготовитель		Дата изготовления
	Использовать до …		Обратитесь к инструкции по применению
	Осторожно! Обратитесь к Инструкции по применению	YYYY-MM-DD YYYY-MM	Дата в формате Год-Месяц-День Год-Месяц

Консультацию специалиста по работе с набором можно получить по тел.: (383) 363-05-97.

12.11.18

АКЦИОНЕРНОЕ ОБЩЕСТВО
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Тел.: (383) 332-37-10, 332-37-58, 332-36-34,
332-67-49, 332-67-52
E-mail: vbmarket@vector-best.ru

www.vector-best.ru

ВЕКТОР БЕСТ

IgM общий-ИФА-БЕСТ

A-8664

Набор реагентов
для иммуноферментного определения
концентрации общего иммуноглобулина
класса М в сыворотке крови

ИНСТРУКЦИЯ ПО ПРИМЕНЕНИЮ

Утверждена 14.08.18

1. НАЗНАЧЕНИЕ

1.1. Набор реагентов для иммуноферментного определения концентрации общего иммуноглобулина класса М в сыворотке крови «IgM общий-ИФА-БЕСТ» (далее по тексту – набор) предназначен для определения концентрации общего иммуноглобулина класса М ($IgM_{общ}$) в сыворотке крови человека методом твердофазного иммуноферментного анализа.

1.2. Набор рассчитан на проведение анализа в дублях 41 неизвестного, 6 калибровочных и 1 контрольного образцов (всего 96 определений при использовании всех стрипов планшета).

2. ХАРАКТЕРИСТИКА НАБОРА

2.1. Принцип метода

Метод определения основан на двухстадийном «сэндвич» – варианте твердофазного иммуноферментного анализа с применением моноклональных антител к IgM.

На первой стадии калибровочные образцы с известной концентрацией $IgM_{общ}$ и анализируемые образцы инкубируются в лунках стрипированного планшета с иммобилизованными моноклональными антителами (МКАТ) к мю-цепям IgM. На второй стадии связавшийся в лунках IgM обрабатывают конъюгатом МКАТ к легким (лямбда и каппа) цепям иммуноглобулинов человека с пероксидазой.

Образовавшиеся иммунные комплексы «иммобилизованные МКАТ – IgM – конъюгат» выявляют ферментативной реакцией с раствором тетраметилбензидина. Степень окрашивания пропорциональна концентрации $IgM_{общ}$ в анализируемом образце. После измерения величины оптической плотности раствора в лунках на основании калибровочного графика рассчитывается концентрация $IgM_{общ}$ в анализируемых образцах.

2.2. Состав набора

В состав набора входят:

- планшет разборный (12 восьмилуночных стрипов) с иммобилизованными на внутренней поверхности лунок моноклональными антителами к мю-цепям IgM человека, готовый для использования – 1 шт.;
- калибровочные образцы, содержащие известные количества $IgM_{общ}$ – 0, 20, 40, 100, 200 и 400 Ед/мл (0; 0,16; 0,32; 0,8; 1,6 и 3,2 мг/мл), аттестованные относительно WHO International Standard Immunoglobulins G, A and M, human serum, NIBSC 67/086; концентрации $IgM_{общ}$ в калибровочных образцах могут несколько отличаться от указанных величин, точные величины указаны на этикетках флаконов, готовые для использования – 6 флаконов (по 0,5 мл);
- контрольный образец на основе инактивированной сыворотки крови человека с известным содержанием $IgM_{общ}$, аттестованный относительно WHO International Standard Immunoglobulins G, A and

M, human serum, NIBSC 67/086; готовый для использования – 1 флакон (0,5 мл);
– конъюгат моноклональных антител к легким цепям (лямбда и каппа) иммуноглобулинов человека с пероксидазой хрена, готовый для использования – 1 флакон (13 мл);
– раствор для разведения сывороток (РРС), концентрат – 1 флакон (28 мл);
– концентрат фосфатно-солевого буферного раствора с твином (ФСБ-Т×25) – 1 флакон (28 мл);
– раствор тетраметилбензидина плюс (раствор ТМБ плюс), готовый для использования – 1 флакон (13 мл);
– стоп-реагент, готовый для использования – 1 флакон (12 мл);
– пленка для заклеивания планшета – 2 шт.;
– ванночка для реагента – 2 шт.;
– наконечники для пипетки на 5–200 мкл – 16 шт.

Принадлежности:

– трафарет для построения калибровочного графика – 1 шт.;
– планшет для предварительного разведения исследуемых образцов – 1 шт.

3. АНАЛИТИЧЕСКИЕ ХАРАКТЕРИСТИКИ

3.1. Специфичность. В наборе «IgM общий-ИФА-БЕСТ» используются моноклональные антитела, обладающие высокой специфичностью к мю-цепям IgM. Перекрестного связывания с IgG, IgA, IgE или альбумином в физиологических концентрациях не наблюдалось.

3.2. «Хук-эффект» при использовании набора реагентов не зафиксирован. Оптическая плотность образцов сыворотки крови с концентрацией $IgM_{общ}$ до 1000 Ед/мл всегда превышала оптическую плотность калибровочного образца с максимальной концентрацией $IgM_{общ}$.

3.3.* Воспроизводимость. Коэффициент вариации результатов определения концентрации $IgM_{общ}$ в лунках, содержащих контрольный образец, не превышает 8%.

3.4.* Линейность. Данный аналитический параметр проверяется тестом на «линейность» – отклонение от расчетной величины концентрации $IgM_{общ}$ при разведении калибровочных образцов, содержащих 400, 200, 100 и 40 Ед/мл, в 2 раза. Процент «линейности» составляет 90–110%.

3.5.* Точность. Данный аналитический параметр проверяется тестом на «открытие» – соответствие измеренной концентрации $IgM_{общ}$ расчетному значению в пробе, полученной путем смешивания равных объемов контрольного образца и калибровочного образца с концентрацией $IgM_{общ}$ 40 Ед/мл. Процент «открытия» составляет 90–110%.

3.6.* Чувствительность. Минимально определяемая концентрация $IgM_{общ}$, рассчитанная на основании среднего арифметического значения оптической плотности калибровочного

* по ГОСТ Р 51352-2013.

образца B_0 (с концентрацией $IgM_{общ}$ 0 Ед/мл) плюс 2σ (σ-среднее квадратичное отклонение от среднего арифметического значения B_0), не превышает 4 Ед/мл (0,032 мг/мл).

3.7. Клиническая проверка. Концентрация $IgM_{общ}$, измеренная в сыворотке крови условно здоровых доноров находилась в диапазоне 60÷270 Ед/мл (см. также стр. 28).

3.8. Рекомендуется в каждой лаборатории при использовании набора уточнить значения концентрации $IgM_{общ}$, соответствующие нормальным для данного региона, у обследуемого контингента людей.

4. МЕРЫ ПРЕДОСТОРОЖНОСТИ

4.1. Потенциальный риск применения набора – класс 2а (приказ МЗ РФ от 06.06.2012 № 4н).

4.2. Все компоненты набора являются нетоксичными. Стоп-реагент обладает раздражающим действием. Избегать разбрызгивания и попадания на кожу и слизистые. В случае попадания стоп-реагента на кожу и слизистые необходимо промыть пораженный участок большим количеством проточной воды.

4.3. При работе с исследуемыми образцами следует соблюдать меры предосторожности, принятые при работе с потенциально инфекционным материалом. Основные правила работы изложены в «Инструкции по мерам профилактики распространения инфекционных заболе-

ваний при работе в клинико-диагностических лабораториях лечебно-профилактических учреждений», утвержденной Минздравом СССР 17 января 1991 г и в методических указаниях МУ 287-113 «Методические указания по дезинфекции, предстерилизационной очистке и стерилизации изделий медицинского назначения», утв. департаментом госсанэпиднадзора Минздрава РФ от 30.12.1998.

4.4. При работе с набором следует надевать одноразовые резиновые или пластиковые перчатки, так как образцы сыворотки крови человека следует рассматривать как потенциально инфицированные, способные длительное время сохранять и передавать ВИЧ, вирусы гепатита или возбудителей других инфекций.

4.5. Лабораторная посуда и оборудование, которые используются в работе с набором, должны быть соответствующим образом промаркированы и храниться отдельно.

4.6. Запрещается прием пищи, использование косметических средств и курение в помещениях, предназначенных для работы с наборами.

4.7. Для дезинфекции посуды и материалов, контактировавших с исследуемыми и контрольными образцами, рекомендуем использовать дезинфицирующие средства, не оказывающие негативного воздействия на качество ИФА, не содержащие активный кислород и хлор, например, комбинированные средства на основе ЧАС,

спиртов, третичных аминов. Использование дезинфицирующих средств, содержащих активный кислород и хлор (H_2O_2, деохлор, хлорамин), приводит к серьезному искажению результатов.

4.8. При использовании набора образуются отходы классов А, Б и Г, которые классифицируются и уничтожаются (утилизируются) в соответствии с СанПиН 2.1.7.2790-10 «Санитарно-эпидемиологические требования к обращению с медицинскими отходами» Дезинфекцию наборов реагентов следует проводить по МУ 287-113 «Методические указания по дезинфекции, предстерилизационной очистке и стерилизации изделий медицинского назначения».

5. ОБОРУДОВАНИЕ И МАТЕРИАЛЫ, НЕОБХОДИМЫЕ ДЛЯ РАБОТЫ С НАБОРОМ:

- спектрофотометр вертикального сканирования, позволяющий проводить измерения оптической плотности растворов в лунках стрипов при основной длине волны 450 нм и длине волны сравнения в диапазоне 620–655 нм; допускается измерение только при длине волны 450 нм;
- шейкер термостатируемый орбитального типа, позволяющий производить встряхивание при температуре 37±1°С и 400–800 об/мин;
- микроцентрифуга, позволяющая центрифугировать при 1500–2000 об/мин;
- промывочное устройство для планшетов;
- холодильник бытовой;

– пипетки полуавтоматические одноканальные с переменным или фиксированным объемом со сменными наконечниками, позволяющие отбирать объемы жидкости от 5 до 5000 мкл;
– пипетка полуавтоматическая многоканальная со сменными наконечниками, позволяющая отбирать объемы жидкостей от 5 до 350 мкл;
– флаконы стеклянные вместимостью 15 мл;
– цилиндр мерный вместимостью 1000 мл;
– вода дистиллированная;
– перчатки медицинские диагностические одноразовые;
– бумага фильтровальная лабораторная;
– дезинфицирующий раствор.

6. АНАЛИЗИРУЕМЫЕ ОБРАЗЦЫ

6.1. Для проведения анализа не следует использовать гемолизованную, мутную сыворотку крови.

6.2. Образцы сыворотки крови можно хранить при температуре от 2 до 8°С не более 48 часов или при температуре минус 20°С (и ниже) не более 3 мес. Повторное замораживание и размораживание образцов сыворотки крови не допускается. После размораживания образцы следует тщательно перемешать.

6.3. Образцы сывороток крови, содержащие осадок, необходимо очистить центрифугированием при 1500 об/мин в течение 10 мин при температуре от 18 до 25°С.

7. ПРОВЕДЕНИЕ АНАЛИЗА

ПОДГОТОВКА РЕАГЕНТОВ

7.1. Перед проведением анализа компоненты набора и исследуемые образцы сыворотки крови следует выдержать при температуре от 18 до 25°С не менее 30 мин.

7.2. Подготовка планшета.

Вскрыть пакет выше замка и установить на рамку необходимое для проведения анализа количество стрипов. Использовать в течение 1 часа после установки. Оставшиеся стрипы немедленно поместить вновь в пакет с влагопоглотителем, удалить из него воздух, плотно закрыть замок.

Хранить при температуре от 2 до 8°С в течение всего срока годности набора.

7.3. Приготовление промывочного раствора

Раствор готовится из концентрата фосфатно-солевого буферного раствора. При выпадении осадка солей в концентрате необходимо прогреть его при температуре от 30 до 40°С до полного растворения осадка.

Внести в мерный цилиндр необходимое количество концентрата фосфатно-солевого буферного раствора с твином (ФСБ-Т×25) и добавить соответствующее количество дистиллированной воды.

В таблице приведен расход реагента в зависимости от количества используемых стрипов.

Приготовленный промывочный раствор можно хранить при температуре от 2 до 8°С не более 5 сут.

7.4. Приготовление рабочего раствора для разведения сывороток.

Приготовить за 30 мин до начала постановки анализа.

При выпадении осадка солей в концентрате РРС необходимо прогреть его при температуре от 30 до 40°С до полного растворения осадка.

Внести в мерный цилиндр необходимое количество концентрата раствора для разведения сывороток и добавить соответствующее количество дистиллированной воды.

В таблице приведен расход реагента в зависимости от количества используемых стрипов.

Приготовленный рабочий раствор для разведения сывороток можно хранить при температуре от 2 до 8°С не более 3 сут.

7.5. Подготовка калибровочных образцов и контрольного образца.

Калибровочные образцы и контрольный образец готовы к использованию и не требуют дополнительного разведения. Перед использованием флаконы встряхнуть или центрифугировать на микроцентрифуге так, чтобы капли растворов со стенок и крышки опустились на

дно. Затем содержимое флаконов тщательно перемешать на вортексе или пипетированием, избегая образования пены.

Калибровочные образцы и контрольный образец после вскрытия можно хранить в плотно закрытых флаконах при температуре от 2 до 8°С в течение всего срока годности набора.

7.6. Приготовление рабочего разведения анализируемых образцов сыворотки крови.

Готовятся в стеклянных заранее промаркированных флаконах за 5–10 мин до начала постановки анализа.

Таблица 1

Кол-во используемых стрипов	Промывочный раствор		Рабочий раствор для разведения сывороток		Конъюгат, мл	Раствор ТМБ плюс, мл
	ФСБ-Т×25, концентрат, мл	Дистил. вода, мл	РРС, концентрат, мл	Дистил. вода, мл		
2	4,0	до 100	4,0	до 100	2,0	2,0
3	6,0	до 150	6,0	до 150	3,0	3,0
4	8,0	до 200	8,0	до 200	4,0	4,0
5	10,0	до 250	10,0	до 250	5,0	5,0
6	12,0	до 300	12,0	до 300	6,0	6,0
7	14,0	до 350	14,0	до 350	7,0	7,0
8	16,0	до 400	16,0	до 400	8,0	8,0
9	18,0	до 450	18,0	до 450	9,0	9,0
10	20,0	до 500	20,0	до 500	10,0	10,0
11	22,0	до 550	22,0	до 550	11,0	11,0
12	24,0	до 600	24,0	до 600	12,0	12,0

В чистый флакон с 10 мл рабочего раствора для разведения сывороток (см п. 7.4) добавить 10 мкл исследуемой сыворотки и тщательно перемешать. Таким образом, рабочее разведение сыворотки составит 1000 раз*.

Использовать в течение 30 мин после приготовления.

7.7. Подготовка конъюгата.

Конъюгат готов к использованию.

Необходимое количество конъюгата отобрать в чистый флакон или ванночку для реагента.

Оставшийся после проведения ИФА конъюгат утилизировать ***(не сливать во флакон с исходным конъюгатом).***

В таблице приведен расход реагента в зависимости от количества используемых стрипов.

Конъюгат после вскрытия можно хранить в плотно закрытом флаконе при температуре от 2 до 8°С в течение всего срока годности набора.

7.8. Подготовка раствора тетраметилбензидина плюс.

Раствор ТМБ плюс готов к использованию.

Необходимое количество раствора ТМБ плюс отобрать в чистый флакон или ванночку для реагента.

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* См. также раздел «Дополнительная информация для потребителей», п. 2

Оставшийся после проведения ИФА раствор ТМБ плюс утилизировать ***(не сливать во флакон с исходным раствором ТМБ плюс).***

Необходимо исключить воздействие прямого света на раствор тетраметилбензидина плюс.

В таблице приведен расход реагента в зависимости от количества используемых стрипов.

Раствор ТМБ плюс после вскрытия можно хранить в плотно закрытом флаконе при температуре от 2 до 8°С в течение всего срока годности набора.

7.9. Стоп-реагент готов к использованию.

После вскрытия можно хранить в плотно закрытом флаконе при температуре от 2 до 8°С в течение всего срока годности набора.

ПРОВЕДЕНИЕ ИФА

7.10. Внести во все лунки по 100 мкл рабочего раствора для разведения сывороток (см п. 7.4).

Внести в соответствующие лунки в дублях, начиная с верхних лунок первых двух стрипов, по 20 мкл каждого калибровочного образца. В следующую пару лунок внести по 20 мкл контрольного образца. В остальные лунки внести в дублях по 20 мкл анализируемых образцов сыворотки крови в рабочем разведении (см п. 7.6.), каждый раз меняя наконечник.

Время внесения образцов не должно превышать 10 мин при использовании всех лунок планшета.

7.11. Планшет заклеить пленкой и инкубировать в течение 20 мин при встряхивании на термостатируемом шейкере при температуре 37±1°С и 700 об/мин.

7.12. По окончании инкубации снять пленку и удалить ее в сосуд с дезинфицирующим раствором. Содержимое лунок удалить отсасыванием в сосуд с дезинфицирующим раствором и промыть, добавляя во все лунки по 350 мкл промывочного раствора (см п. 7.3.). Процесс промывки повторить еще 4 раза. Общее количество отмывок равно 5. Время между заполнением и опорожнением лунок должно быть не менее 30 сек. Необходимо следить за полным опорожнением лунок после каждого цикла отмывки. Затем удалить остатки жидкости из лунок, постукивая планшетом в перевернутом положении по фильтровальной бумаге.

7.13. Внести во все лунки планшета по 100 мкл конъюгата (см п. 7.7.).

Для внесения конъюгата использовать ванночку для реагента и одноразовые наконечники, входящие в состав набора.

7.14. Планшет заклеить пленкой и инкубировать в течение 20 мин при встряхивании на термостатируемом шейкере при температуре 37±1°С и 700 об/мин.

7.15. По окончании инкубации удалить содержимое лунок и промыть планшет, как это указано в п. 7.12.

7.16. Внести во все лунки по 100 мкл раствора тетраметилбензидина плюс (см п. 7.8.) и инкубировать в защищенном от света месте в течение 15 мин при температуре от 18 до 25°С.

Для внесения раствора тетраметилбензидина плюс использовать ванночку для реагента и одноразовые наконечники, входящие в состав набора.

7.17. Внести во все лунки с той же скоростью и в той же последовательности, как и раствор тетраметилбензидина плюс, по 100 мкл стоп-реагента; при этом содержимое лунок окрашивается в желтый цвет.

8. РЕГИСТРАЦИЯ РЕЗУЛЬТАТОВ

Измерить величину оптической плотности растворов в лунках стрипов на спектрофотометре вертикального сканирования в двухволновом режиме: основной фильтр – 450 нм, референс-фильтр в диапазоне 620–655 нм; допускается измерение только с фильтром 450 нм. Измерение проводить через 2–3 мин после остановки реакции.

Время между остановкой реакции и измерением оптической плотности не должно превышать 10 мин.

9. УЧЕТ РЕЗУЛЬТАТОВ

9.1. Вычислить средние арифметические значения оптической плотности для каждой пары лунок, содержащих калибровочные образцы.

9.2. Построить в линейных координатах калибровочный график зависимости среднего арифметического значения оптической плотности (ед. опт. плотн.) от концентрации $IgM_{общ}$ в калибровочных образцах (Ед/мл или мг/мл).

9.3. Определить концентрацию $IgM_{общ}$ в контрольном образце и анализируемых образцах по калибровочному графику. Вычислить среднее арифметическое значение концентрации для каждой пары лунок, содержащих анализируемые образцы.

9.4. Если при проведении анализа использовали разведение сыворотки в 1000 раз (базовое разведение для данного набора), то найденное по графику количество $IgM_{общ}$ соответствует концентрации $IgM_{общ}$ в анализируемом образце в Ед/мл (мг/мл). Если использовали другое разведение образца, то найденное по графику количество $IgM_{общ}$ пересчитывают с учетом дополнительного разведения, также получая в результате концентрацию $IgM_{общ}$ в Ед/мл (мг/мл).

Если значение оптической плотности анализируемого образца превышает значение ОП для калибровочного образца 400 Ед/мл (3,2 мг/мл), то данный образец анализируют повторно после дополнительного разведения в 2 раза, полученный результат умножают на 2.

10. УСЛОВИЯ ТРАНСПОРТИРОВАНИЯ, ХРАНЕНИЯ И ПРИМЕНЕНИЯ НАБОРА

10.1. Транспортировать изделия следует транспортом всех видов в крытых транспортных средствах в соответствии с правилами перевозок, действующими на транспорте данного вида, при температуре от 2 до 8°С. Допускается транспортирование при температуре до 25°С не более 10 суток.

10.2. Хранение набора в упаковке предприятия-изготовителя должно осуществляться при температуре от 2 до 8°С в течение всего срока годности в холодильных камерах или холодильниках, обеспечивающих регламентированный температурный режим с ежедневной регистрацией температуры.

10.3. Срок годности набора – 12 месяцев со дня выпуска. Не допускается применение наборов по истечении срока их годности.

10.4. Дробное использование набора может быть реализовано в течение всего срока годности. В случае дробного использования набора:

– неиспользованные стрипы можно хранить в плотно закрытом пакете при температуре от 2 до 8°С в течение всего срока годности;
– калибровочные образцы, контрольный образец и конъюгат после вскрытия можно хранить в плотно закрытых флаконах при температуре от 2 до 8°С в течение всего срока годности набора;
– концентрат фосфатно-солевого буферного раствора с твином, концентрат раствора для разведения сыво-

роток, раствор ТМБ плюс и стоп-реагент после вскрытия можно хранить в плотно закрытых флаконах при температуре от 2 до 8°С в течение всего срока годности набора;

– рабочий раствор для разведения сывороток можно хранить при температуре от 2 до 8°С не более 3 сут;
– промывочный раствор можно хранить при температуре от 2 до 8°С не более 5 сут.

10.5. Построение калибровочного графика необходимо проводить для каждого независимого эксперимента, рекомендуется также каждый раз определять концентрацию $IgM_{общ}$ в контрольном образце.

10.6. Для перевода результатов измерений концентрации иммуноглобулина М из Ед/мл в мг/мл следует использовать коэффициент пересчета 0,008 (1 Ед/мл IgM = 0,008 мг/мл IgM).

10.7. При постановке ИФА нельзя использовать компоненты из наборов разных серий или смешивать их при приготовлении растворов, кроме неспецифических компонентов (раствор ТМБ плюс, ФСБ-Т×25, стоп-реагент), которые взаимозаменяемы во всех наборах АО «Вектор-Бест».

10.8. Для получения надежных результатов необходимо строгое соблюдение инструкции по применению набора.

11. ГАРАНТИЙНЫЕ ОБЯЗАТЕЛЬСТВА

11.1. Производитель гарантирует соответствие выпускаемых изделий требованиям нормативной и технической документации.

Безопасность и качество изделия гарантируются в течение всего срока годности.

11.2. Производитель отвечает за недостатки изделия, за исключением дефектов, возникших вследствие нарушения правил пользования, условий транспортирования и хранения, либо действия третьих лиц, либо непреодолимой силы.

11.3. Производитель обязуется за свой счет заменить изделие, технические и функциональные характеристики (потребительские свойства) которого не соответствуют нормативной и технической документации, если указанные недостатки явились следствием скрытого дефекта материалов или некачественного изготовления изделия производителем.

По вопросам, касающимся качества набора «IgM общий-ИФА-БЕСТ», следует обращаться в АО «Вектор-БЕСТ» по адресу:
630559, Новосибирская область,
Новосибирский район, п. Кольцово, а/я 121,
тел. (383) 363-20-60, 227-75-43,
тел./факс (383) 363-35-55.
E-mail: vbobtk@vector-best.ru

ДОПОЛНИТЕЛЬНАЯ ИНФОРМАЦИЯ ДЛЯ ПОТРЕБИТЕЛЕЙ.

Набор предназначен для профессионального применения в клинической лабораторной диагностике обученным персоналом.

Требования безопасности к медицинским лабораториям приведены в ГОСТ Р 52905-2007.

Все реагенты наборов, содержащие в своем составе материалы человеческого происхождения, инактивированы.

При динамическом наблюдении пациента для получения результатов, адекватно отражающих изменение концентрации $IgM_{общ}$ в крови, необходимо использовать наборы реагентов одного наименования (одного предприятия-изготовителя).

1. Обеспечение получения правильных результатов анализа

Достоверность и воспроизводимость результатов анализа зависят от выполнения следующих основных правил:

– не проводите ИФА в присутствии паров кислот, щелочей, альдегидов или пыли, которые могут влиять ферментативную активность конъюгатов;

– ферментативная реакция чувствительна к присутствию ионов металлов, поэтому не допускайте контактов каких-либо металлических предметов с конъюгатом и раствором ТМБ;

– избегайте загрязнения компонентов набора микроорганизмами и химическими примесями, для этого используйте в работе чистую посуду и чистые одноразовые наконечники для каждого реагента, контроля, образца;

– рабочие поверхности столов, оборудования обрабатывайте 70% этиловым спиртом (не допускается использование перекиси водорода, хлорсодержащих растворов);

– никогда не используйте одну и ту же емкость для конъюгата и раствора ТМБ;

– перед отбором ТМБ из флакона необходимо обрабатывать конус пипетки (внутреннюю и внешнюю поверхности) сначала дистиллированной водой, а затем 70% этиловым спиртом, так как малейшее загрязнение пипеток конъюгатом может привести к контаминации всего содержимого флакона с ТМБ;

– если допущена ошибка при внесении в лунку анализируемого образца, нельзя, опорожнив эту лунку, вносить в нее новый образец; такая лунка бракуется.

Качество промывки лунок планшета играет важную роль для получения правильных результатов анализа:

– Для аспирации анализируемых образцов и последующей промывки используйте автоматическое или ручное промывочное устройство.

– Не допускайте высыхания лунок планшета в перерыве между завершением промывки и внесением реагентов.

– Добивайтесь полного заполнения и опорожнения всех лунок планшета в процессе промывки. Недостаточная аспирация жидкости в процессе промывки может привести к понижению чувствительности и специфичности анализа.

– Следите за состоянием промывочного устройства – регулярно (1 раз в неделю) обрабатывайте шланги и емкости 70% этиловым спиртом.

– Для предотвращения засорения игл промывочного устройства в конце рабочего дня обязательно выполните процедуру ополаскивания системы подачи жидкости дистиллированной водой.

2. Рекомендации по подготовке анализируемых образцов

Вместо одноступенчатого (п. 7.6.) допустимо проводить двухступенчатое разведение сывороток с использованием планшета для предварительного разведения исследуемых образцов. Для этого, в каждую лунку планшета для предварительного разведения внести по 310 мкл рабочего раствора для разведения сывороток. Далее в одну из лунок, например, А-1, добавить 10 мкл исследуемой сыворотки, сменить использованный наконечник пипетки на новый и затем с его помощью тщательно перемешать содержимое лунки (5–6 круговых движений, во время которых следует 3–4 раза набрать и опорожнить наконечник), избегая образования пены. После

этого из лунки отобрать 10 мкл, внести в соседнюю лунку, например, А-2, и таким же образом тщательно перемешать (для этой операции также желательно использовать новый чистый наконечник пипетки). В лунке А-2 получаем рабочее разведение сыворотки 1000 раз. Аналогично развести и другие исследуемые сыворотки (например, в лунках В-1 и В-2, С-1 и т.д.):

– 310 мкл рабочего раствора для разведения сывороток + 10 мкл исследуемого образца → предварительное разведение образца в 32 раза;

– 310 мкл рабочего раствора для разведения сывороток + 10 мкл образца после предварительного разведения → рабочее разведение образца в 1000 раз.

Внимание! *Точность приготовления разведений определяет качество постановки теста!*

При исследовании не сыворотки, а других биологических жидкостей, степень разведения исследуемых образцов следует заранее подобрать опытным путем, используя как ориентир данные таблицы 2.

3. Условия правильности работы набора

Результаты анализа исследуемых образцов учитывать, если будут выполнены следующие условия:

– соотношение оптических плотностей калибровочных образцов:

$ОП_0 < ОП_{20} < ОП_{40} < ОП_{100} < ОП_{200} < ОП_{400}$;

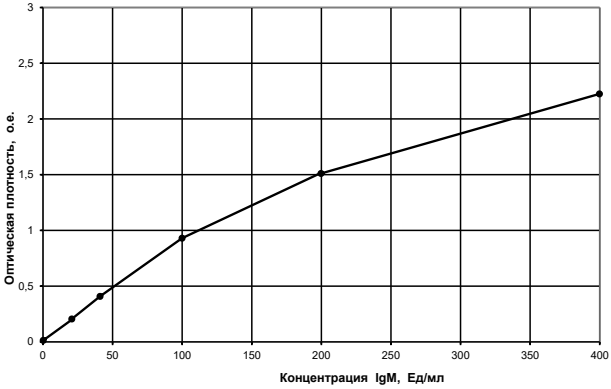

Рисунок. Зависимость оптической плотности от концентрации $IgM_{общ}$ в калибровочных образцах.

– $ОП_{400} \geq 1{,}0$ ед. опт. плотн. (о.е.);

– вычисленное по калибровочному графику значение концентрации $IgM_{общ}$ в контрольном образце попадает в пределы, указанные на этикетке флакона.

$ОП_0$, $ОП_{20}$, $ОП_{40}$, $ОП_{100}$, $ОП_{200}$ и $ОП_{400}$ – среднее значение оптической плотности калибровочных образцов 0; 20; 40; 100; 200 и 400 мг/мл соответственно.

4. Расчет результатов анализа

По результатам измерения вычислить среднее арифметическое значение оптической плотности (ОП) в лунках с анализируемыми образцами.

Построить в линейных координатах калибровочный график зависимости оптической плотности (ось ординат) от концентрации $IgM_{общ}$ (ось абсцисс) в калибровочных образцах. Для этого на прилагаемом трафарете для построения графика против концентрации каждого калибровочного образца отложить соответствующее ей среднее значение оптической плотности. Последовательно соединить полученные точки отрезками прямых линий.

Пример калибровочного графика представлен на рисунке.

Определить содержание $IgM_{общ}$ в контрольном и в анализируемых образцах по калибровочному графику. Для этого на оси ординат отметить значение ОП анализируемого образца. Провести прямую линию параллельно оси абсцисс до пересечения с калибровочным графиком. От точки пересечения опустить перпендикуляр на ось абсцисс. По полученной точке пересечения определить значение концентрации $IgM_{общ}$ в образце.

При использовании для расчетов концентраций компьютерного или встроенного в спектрофотометр программного обеспечения в настройках выбрать метод, соответствующий кусочно-линейной аппроксимации.

5. Диагностическая значимость

IgM, как и другие иммуноглобулины, относится к гуморальным факторам иммунитета.

Карта гуморального иммунитета довольно индивидуальна, тем не менее, пределы нормальных физиологических концентраций достаточно хорошо очерчены.

По нашим данным, концентрация $IgM_{общ}$ в сыворотке крови клинически здоровых доноров (Новосибирская область, Алтайский край) в возрасте от 20 до 50 лет находится в пределах диапазона 60–250 Ед/мл (0,48–2,0 мг/мл). У детей того же региона в возрасте 1–15 лет концентрация $IgM_{общ}$ составила 60–270 Ед/мл (0,48–2,16 мг/мл). Эти значения в целом близки нормальным значениям концентрации IgM (0,5–2,0 мг/мл), приводимым в работе Тотолян А.А., Марфичева Н.А., Тотолян Н.А. «Иммуноглобулины в клинической лабораторной диагностике», С-Пб, 1999. Однако их можно использовать только как ориентировочные, поскольку диапазоны нормальных концентраций IgM могут довольно существенно отличаться в зависимости от региона, возраста и некоторых др. причин. Известно также, что для использования в диагностике важнее знать не абсолютное значение концентрации общего иммуноглобулина, а его относительное отклонение от нормального местного, возрастного или, например, профессионального уровня. **В идеале, нормальные региональные уровни и по взрослым и по детям должны определяться каждой лабораторией самостоятельно!**

Таблица 2

Абсолютные значения уровней содержания иммуноглобулинов в различных биологических жидкостях у здоровых лиц (M±δ)

(Тотолян А.А. Современные подходы к диагностике иммунопатологических состояний. Мед. иммунология, 1999 ,т.1 №1-2, с. 75-108)

Биологические жидкости	Содержание иммуноглобулинов классов:				
	***IgA*, г/л**	***IgM*, г/л**	***IgG*, г/л**	***sIgA*, г/л**	***IgE*, кЕ/л**
ЦСЖ	0,006±0,0013	0,0049±0,001	0,037±0,004	н/опр	0±0
Слюна	0,069±0,028	0,055±0,011	0,042±0,017	0,768±0,275	н/опр
Назальный смыв	0,014±0,006	0,025±0,017	0,042±0,017	0,071±0,022	0±0
Ларингеальный секрет	0,071±0,022	0,063±0,044	0,085±0,044	1,31±1,87	н/опр
Слезная жидкость	0,165±0,02	0,038±0,008	0,185±0,06	н/опр	н/опр
Эякулят	1,01±0,67	0,9±0,46	0,51±0,2	2,21±1,01	0±0
Сыворотка крови	2,15±0,85	1,63±0,46	12,3±2,97	0,79±0,22	50,0±12,5

Примечание: н/опр – данный показатель не определяли.

Приведенные показатели можно использовать только как ориентировочные, и в каждой лаборатории рекомендуется вычислить собственные границы нормальных значений концентрации общего IgM в сыворотке крови.

Уровень концентрации общего IgM в сыворотке крови новорожденных составляет около 10% от уровня взрослых. Через 1–3 месяца он увеличивается до 60-65%, а еще через 1–2 года нередко уже достигает уровня взрослого человека. Однако в этом возрасте возможны его значительные колебания. У подавляющего же большинства здоровых детей уровень концентрации общего IgM стабилизируется и становится равным уровню взрослых в возрасте 6–8 лет.

Результаты определения концентрации общего сывороточного IgM могут быть с успехом использованы для дифференциальной диагностики целого ряда заболеваний (см. иммунограмму).

Более полную картину способно дать параллельное определение всех трех основных классов иммуноглобулинов – G, M и A, а также иммуноглобулина E.

Иммунограмма при некоторых заболеваниях

	IgG	IgA	IgM	IgE
Заболевания печени				
Острый инфекционный гепатит	+	N/+	N/++	N
Хронический персистирующий гепатит	N/+	N	N/+	N/+
Хронический агрессивный гепатит	++	+	N/++	N/+
Постгепатитный криптогенный цирроз	++	+	+	N/+
Первичный билиарный цирроз	N/+	N	+/++	N
Алкогольный цирроз	N/+	++	N/+	N
Болезни почек				
Острый пиелонефрит	N	N	+/++	N
Хронический пиелонефрит	+/++	N	+/++	N/+
Нефротический синдром	—	—	N/—	N/—
Инфекционные заболевания				
Острая инфекция	N	N	+/++	N
Хроническая инфекция	+/++	N/+	N/+	N/+
Системные ревматические заболевания				
Ревматоидный артрит	N/++	N/++	N/+	+/++
Системная красная волчанка	+	N	N/+	N/+
Склеродермия	N	N	N	N/+
Смешанные системные заболевания	N/+	N/+	N	N/+
Атопия, аллергические заболевания	N/+	N	N/—	+/++
Гельминтозы и др. паразитарные заболевания	N/+	N/+	N/+	+/++

N – нормальная регионально-возрастная концентрация иммуноглобулина (в пределах нормального диапазона от Nmin до Nmax)

\+ – повышенная концентрация иммуноглобулина (от Nmax до 1,3Nmax)

++ – сильно повышенная концентрация иммуноглобулина (более 1,3Nmax)

– – пониженная концентрация иммуноглобулина (ниже Nmin)

6. Краткая схема проведения ИФА для набора реагентов «IgM общий-ИФА-БЕСТ»

Использовать только после внимательного ознакомления с инструкцией!

Внести:	по 100 мкл рабочего раствора для разведения сывороток;
	по 20 мкл калибровочных и контрольного образцов в дублях в контрольные лунки;
	по 20 мкл разведенных анализируемых образцов в дублях в лунки для исследуемых образцов.
Инкубировать:	20 мин, 37°С, 700 об/мин.
Промыть:	промывочный раствор, 350 мкл, 5 раз.
Внести:	по 100 мкл конъюгата.
Инкубировать:	20 мин, 37°С, 700 об/мин.
Промыть:	промывочный раствор, 350 мкл, 5 раз.
Внести:	по 100 мкл раствора ТМБ плюс.
Инкубировать:	15 мин, 18–25°С, в темноте.
Внести:	по 100 мкл стоп-реагента.
Измерить:	ОП при 450 нм / референсная длина волны 620–655 нм.

7. Графические символы

REF	Номер по каталогу	IVD	Медицинское изделие для диагностики *in vitro*
Σ n	Содержимого достаточно для проведения n-количества тестов	NON STERILE	Не стерильно
LOT	Код партии		Температурный диапазон
	Изготовитель		Дата изготовления
	Использовать до …		Обратитесь к инструкции по применению
	Осторожно! Обратитесь к Инструкции по применению	YYYY-MM-DD YYYY-MM	Дата в формате Год-Месяц-День Год-Месяц

Консультацию специалиста по работе с набором можно получить по тел.: (383) 363-05-97.

12.11.18

АКЦИОНЕРНОЕ ОБЩЕСТВО
«ВЕКТОР-БЕСТ»

Международный сертификат ISO 13485

Наш адрес: 630117, Новосибирск-117, а/я 492
Тел./факс: (383) 227-73-60 (многоканальный)
Тел.: (383) 332-37-10, 332-37-58, 332-36-34,
332-67-49, 332-67-52
E-mail: vbmarket@vector-best.ru

www.vector-best.ru

MULTI LIGAND CONTROL-TRI LEVEL **PRODUCT CODE: ML-300B**

LOT# MLAC1D3 **EXP: 2026-04-18**

INTENDED USE

The Multi-ligand Controls are intended for use as an assayed quality control material to monitor the consistency of performance of laboratory test procedures associated with determination and monitoring of the clinical status. This product is a human-serum based, lyophilized control, stabilized with preservatives and can be used with all ELISA and CLIA methods.

SUMMARY AND EXPLANATION

The use of quality control material to assist in the assessment of precision in the clinical laboratory is an integral part of laboratory practices. Controls that contain varied levels of analytes are necessary to insure precision and accuracy in immunoassay systems.

REAGENTS

Monobind's Multi-ligand Controls are intended to be used in the exact manner as patient samples. The control is packaged as 6 vials of 3.0 ml, dried. The analyte activities are adjusted to concentrations in the low, middle and high range in order to monitor the efficacy of the procedure in use.

INSTRUCTIONS FOR USE

1) Bring the vials to room temperature before use.
2) Carefully unscrew and remove cap.
3) Add three (3) ml of distilled or deionized water to each vial. Close the cap tightly and let the contents mix thoroughly for 30 minutes
4) Aliquot the materials in 0.5 ml aliquots in cryo vials and store at -20ºC.

STORAGE, STABILITY AND DISPOSAL

This product will be stable until the expiration date when stored unopened at 2 to 8°C. Once the control is reconstituted, all analytes will be stable for 7 days when stored tightly capped at 2 to 8°C with the following exceptions: 1) **C-Peptide, f-PSA, and PRL** should be assayed immediately after reconstitution, and 2) **Folate, Insulin, and PRL-Seq** will be stable for 1 day. To avoid contamination, it is recommended labs aliquot required quantities into vials before each use.

After reconstituting, controls should be tightly capped and returned to refrigerator 2 to 8° C as soon as practical after usage. (Long term room temperature storage is not supported.) After reconstituting, controls should be tightly capped and frozen within 2-hours. **Once thawed, do not refreeze the control; discard remaining materia**l. It is recommended that customers aliquot control into separate containers before freezing to allow for usage on different days. Outdated material should be discarded as a biohazardous component.

STORAGE	STABILITY	TEMPERATURE
Lyophilized, Unopened	Three (3) years	< 8°C
Reconstituted, Opened	Seven (7) days	2 - 8°C
Reconstituted, Opened	Ninety (90) days	< -10°C

EXPECTED RANGE OF VALUES

The mean values printed in this insert were derived from replicate analyses and are specific for this lot of product. The tests listed were performed by Monobind QA using representative lots of this product, as well as those of Monobind's AccuBind® ELISA and AccuLite® CLIA reagents.

Individual laboratory means should fall within the corresponding acceptable range; however laboratory means may vary from the listed values during the life of this control. Therefore, each laboratory should establish its own means and acceptable ranges for the product used, using Monobind's assignment only as guide. A trend log should be maintained for batch to batch consistency of the test. Variations over time and between laboratories may be caused by a) differences in laboratory personnel, b) improper technique, c) instrumentation and reagents, d) improper dilutions from the stated manufacturer's procedure, and/ or e) modifications in the manufacturer's test procedure.

Refer to http://www.monobind.com/site/qc-documents.html for any updated insert information.

WARNING AND PRECAUTIONS

FOR IN VITRO DIAGNOSTIC USE

All products that contain human serum have been found to be non-reactive for HIV 1&2, HIV-Ag, HBsAg, HCV and RPR by FDA required tests. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Edition, 1988, HHS Publication No. (CDC) 88-8395.

Revision: 1 **Date: 2023-06-15** **Product Code: ML-300B**

For Orders and Inquires, please contact

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Please visit our website to learn more about our products and services.

Glossary of Symbols
(EN 980/ISO 15223)

IVD — In Vitro - Diagnostic Medical Device

Temperature Limitation Storage Condition (2-8° C)

Consult Instructions for Use

REF — Catalogue Number

Used By (Expiration Day)

LOT — Batch Code

Date of Manufacturer

Manufacturer

EC REP — Authorized Rep in European Country

CE 0050 — European Conformity NB Id #

DOCUMENT HISTORY

PREPARED BY: _____ DEPT: QC VERIFIED BY: AShatoka DEPT:QA

APPROVED BY: _____ DEPT: Admin EFFECTIVE DATE: 2023-06-15

REVISION: 1 DCO: 1624

EXPECTED RANGE OF VALUES FOR MULTI-LIGAND CONTROL - TRI LEVEL

MASTER LOT: MLAC1D3

Analyte	A Range	B Range	C Range	Method
Allergy				
IgE in IU/ml	99.49 ± 32.83	23.24 ± 7.67	158.73 ± 52.38	MB ACCUBIND ELISA
	93.38 ± 30.81	20.30 ± 6.70	159.85 ± 52.75	MB ACCULITE CLIA
Anemia				
Ferritin in ng/ml	27.79 ± 9.17	81.75 ± 26.98	333.07 ± 109.91	MB ACCUBIND ELISA
	24.94 ± 8.23	82.13 ± 27.10	364.67 ± 120.34	MB ACCULITE CLIA
Folate in ng/ml	1.85 ± 0.61	7.90 ± 2.61	12.84 ± 4.24	MB ACCUBIND ELISA
	2.45 ± 0.81	8.58 ± 2.83	13.53 ± 4.46	MB ACCULITE CLIA
Vitamin B12 in pg/ml	286.89 ± 94.67	414.64 ± 136.83	1032.44 ± 340.70	MB ACCUBIND ELISA
	330.71 ± 109.13	428.07 ± 141.26	1021.96 ± 337.25	MB ACCULITE CLIA
Anemia VAST				
(Folate) in ng/ml	2.71 ± 0.89	7.83 ± 2.58	12.49 ± 4.12	MB ACCUBIND ELISA
	2.78 ± 0.92	7.76 ± 2.56	11.62 ± 3.84	MB ACCULITE CLIA
(Vitamin B12) in pg/ml	366.57 ± 120.97	466.13 ± 153.82	978.22 ± 322.81	MB ACCUBIND ELISA
	346.20 ± 114.25	469.55 ± 154.95	919.75 ± 303.52	MB ACCULITE CLIA
Bone Metabolism				
Vit D Direct in ng/ml	28.57 ± 9.43	46.66 ± 15.40	88.77 ± 29.29	MB ACCUBIND ELISA
	31.25 ± 10.31	47.51 ± 15.68	141.73 ± 46.77	MB ACCULITE CLIA
Cancer Markers				
AFP in ng/ml	20.70 ± 6.83	86.38 ± 28.51	190.16 ± 62.75	MB ACCUBIND ELISA
	20.22 ± 6.67	91.03 ± 30.04	195.92 ± 64.65	MB ACCULITE CLIA
CEA in ng/ml	4.02 ± 1.33	19.12 ± 6.31	45.45 ± 15	MB ACCUBIND ELISA
	3.98 ± 1.31	18.12 ± 5.98	48.52 ± 16.01	MB ACCULITE CLIA
CEA Next Generation in ng/ml	4.28 ± 1.41	24.29 ± 8.02	73.41 ± 24.23	MB ACCUBIND ELISA
	3.85 ± 1.27	22.94 ± 7.57	66.64 ± 21.99	MB ACCULITE CLIA
fPSA in ng/ml	0.77 ± 0.25	3.03 ± 1	> 10	MB ACCUBIND ELISA
	0.79 ± 0.26	3.40 ± 1.12	> 10	MB ACCULITE CLIA
tPSA-XS in ng/ml	1.10 ± 0.36	3.63 ± 1.20	23.03 ± 7.60	MB ACCUBIND ELISA
	1 ± 0.33	3.59 ± 1.18	22.99 ± 7.59	MB ACCULITE CLIA
tPSA in ng/ml	1.35 ± 0.44	4.38 ± 1.44	25.63 ± 8.46	MB ACCUBIND ELISA
	1.13 ± 0.37	4.07 ± 1.34	24.77 ± 8.18	MB ACCULITE CLIA
Cancer Markers VAST				
(CEA) in ng/ml	3.70 ± 1.22	18.45 ± 6.09	45.61 ± 15.05	MB ACCUBIND ELISA
	3.33 ± 1.10	16.74 ± 5.53	46.28 ± 15.27	MB ACCULITE CLIA
(AFP) in ng/ml	20.81 ± 6.87	92.04 ± 30.37	189.19 ± 62.43	MB ACCUBIND ELISA
	19.70 ± 6.50	82.27 ± 27.15	184.37 ± 60.84	MB ACCULITE CLIA
(tPSA) in ng/ml	1.23 ± 0.40	4.29 ± 1.42	30.77 ± 10.16	MB ACCUBIND ELISA
	1.08 ± 0.36	4.24 ± 1.40	29.32 ± 9.68	MB ACCULITE CLIA
Cardiac Markers				
Dig in ng/ml	0.36 ± 0.12	1.69 ± 0.56	2.68 ± 0.88	MB ACCUBIND ELISA
	0.46 ± 0.15	1.61 ± 0.53	2.84 ± 0.94	MB ACCULITE CLIA
Diabetes				
C-Peptide in ng/ml	0.48 ± 0.16	2.38 ± 0.79	4.56 ± 1.50	MB ACCUBIND ELISA
	0.44 ± 0.15	2.29 ± 0.75	4.19 ± 1.38	MB ACCULITE CLIA
Insulin in µIU/ml	28.97 ± 9.56	82.75 ± 27.31	169.78 ± 56.03	MB ACCUBIND ELISA
	26.90 ± 8.88	84.63 ± 27.93	162.90 ± 53.76	MB ACCULITE CLIA
Rapid Insulin in µIU/ml	28.02 ± 9.25	81.63 ± 26.94	159.35 ± 52.59	MB ACCUBIND ELISA
Fertility				
FSH in mIU/ml	8.64 ± 2.85	24.11 ± 7.96	42.71 ± 14.09	MB ACCUBIND ELISA
	7.92 ± 2.61	23.58 ± 7.78	41.52 ± 13.70	MB ACCULITE CLIA
hCG in mIU/ml	4.43 ± 1.46	24.10 ± 7.95	146.28 ± 48.27	MB ACCUBIND ELISA
	4.35 ± 1.74	22.95 ± 7.57	151.35 ± 49.95	MB ACCULITE CLIA
hCG-XR in mIU/ml	4.18 ± 1.38	28.67 ± 9.46	143.88 ± 47.48	MB ACCUBIND ELISA
	3.56 ± 1.17	28.58 ± 9.43	155.85 ± 51.43	MB ACCULITE CLIA
LH in mIU/ml	3.88 ± 1.28	22.25 ± 7.34	53.53 ± 17.67	MB ACCUBIND ELISA
	3.38 ± 1.12	20.07 ± 6.62	53.08 ± 21.72	MB ACCULITE CLIA
PRL in ng/ml	5.08 ± 1.68	24.36 ± 8.04	38.50 ± 12.70	MB ACCUBIND ELISA
	4.78 ± 1.58	21.14 ± 6.98	36.80 ± 12.14	MB ACCULITE CLIA
PRL-seq in ng/ml	4.24 ± 1.41	20.16 ± 6.65	35.96 ± 11.87	MB ACCUBIND ELISA
	4.14 ± 1.37	18.63 ± 6.15	36.61 ± 12.08	MB ACCULITE CLIA
Rapid HCG in mIU/ml	4.70 ± 1.55	27.56 ± 9.10	188.52 ± 62.21	MB ACCUBIND ELISA
Fertility VAST				
(FSH) in mIU/ml	6.84 ± 2.26	18.59 ± 6.13	32.98 ± 10.88	MB ACCUBIND ELISA
	6.12 ± 2.02	17.63 ± 5.82	37 ± 12.21	MB ACCULITE CLIA
(LH) in mIU/ml	4.29 ± 1.42	22.36 ± 7.38	50.17 ± 16.56	MB ACCUBIND ELISA
	3.74 ± 1.23	20.37 ± 6.72	43.9 ± 14.49	MB ACCULITE CLIA
(hCG) in mIU/ml	4.88 ± 1.61	24.08 ± 7.95	144.63 ± 47.73	MB ACCUBIND ELISA
	5.92 ± 1.95	26.53 ± 8.76	149.81 ± 49.44	MB ACCULITE CLIA
Triple Screen VAST				
(AFP) in ng/ml	21.47 ± 7.09	103.20 ± 34.05	188.63 ± 62.25	MB ACCUBIND ELISA
	19.13 ± 6.31	100.30 ± 33.10	203.38 ± 67.11	MB ACCULITE CLIA
(uE3) in ng/ml	1.11 ± 0.37	3.32 ± 1.10	5.99 ± 1.98	MB ACCUBIND ELISA
	1.10 ± 0.36	2.72 ± 0.90	5.40 ± 1.78	MB ACCULITE CLIA
(hCG) in mIU/ml	4.29 ± 1.41	23.43 ± 7.73	149.04 ± 49.18	MB ACCUBIND ELISA
	4.78 ± 1.58	21.30 ± 7.03	174.85 ± 57.70	MB ACCULITE CLIA
Growth Deficiency				
hGH in µIU//ml	5.29 ± 1.75	32.33 ± 10.67	67.95 ± 22.42	MB ACCUBIND ELISA
	5.01 ± 1.65	32.03 ± 10.57	68 ± 23	MB ACCULITE CLIA
Steroids				
Aldosterone in ng/ml	51.72 ± 17.43	471.16 ± 155.48	1195.18 ± 394.41	MB ACCUBIND ELISA
	60.35 ± 19.92	447.70 ± 147.74	1167.75 ± 385.36	MB ACCULITE CLIA
ANST in ng/ml	1 ± 0.33	1.52 ± 0.50	10.60 ± 3.50	MB ACCUBIND ELISA
	0.89 ± 0.29	1.36 ± 0.45	11.45 ± 3.78	MB ACCULITE CLIA
Cortisol in µg/dl	2.43 ± 0.80	13.98 ± 4.61	30.98 ± 11.40	MB ACCUBIND ELISA
	3.02 ± 1	14.91 ± 4.92	33.37 ± 11.01	MB ACCULITE CLIA
DHEA-S in µg/ml	0.37 ± 0.12	1.64 ± 0.54	4.40 ± 1.45	MB ACCUBIND ELISA
	0.40 ± 0.17	1.51 ± 0.50	3.99 ± 1.32	MB ACCULITE CLIA
DHEA in ng/ml	0.89 ± 0.30	2.94 ± 0.97	12.42 ± 4.10	MB ACCUBIND ELISA
	1.02 ± 0.34	3.34 ± 1.10	14.14 ± 4.67	MB ACCULITE CLIA
E1 in ng/ml	32 ± 13.02	149.61 ± 49.37	365.28 ± 120.54	MB ACCUBIND ELISA
E2 in pg/ml	36.26 ± 11.96	180.72 ± 59.64	295.83 ± 97.62	MB ACCUBIND ELISA
	35.85 ± 11.83	189 ± 62.37	283.05 ± 93.41	MB ACCULITE CLIA
uE3 in ng/ml	1.04 ± 0.41	2.43 ± 0.80	5.14 ± 1.70	MB ACCUBIND ELISA
	1.19 ± 0.39	2.51 ± 0.83	4.97 ± 1.64	MB ACCULITE CLIA
Progesterone in ng/ml	0.97 ± 0.33	7.20 ± 2.37	25.05 ± 8.27	MB ACCUBIND ELISA
	1.01 ± 0.33	7.10 ± 2.34	25.39 ± 8.38	MB ACCULITE CLIA
17-OHP in ng/ml	0.62 ± 0.20	2.01 ± 0.66	5.67 ± 1.87	MB ACCUBIND ELISA
	0.71 ± 0.24	2.07 ± 0.68	5.71 ± 1.89	MB ACCULITE CLIA
17-OHP-SI in ng/ml	0.36 ± 0.12	1.13 ± 0.37	3 ± 0.99	MB ACCUBIND ELISA
	0.4 ± 0.13	1 ± 0.33	2.90 ± 0.96	MB ACCULITE CLIA
Testosterone in ng/ml	0.28 ± 0.09	1.03 ± 0.34	6.93 ± 2.29	MB ACCUBIND ELISA
	0.42 ± 0.14	0.90 ± 0.30	7.93 ± 2.62	MB ACCULITE CLIA
Free Testosterone (0-60pg/ml calibration)	1.11 ± 0.37	3.46 ± 1.14	28.89 ± 9.53	MB ACCUBIND ELISA
	1.21 ± 0.40	3.69 ± 1.22	31.29 ± 10.32	MB ACCULITE CLIA
Thyroid				
T3 in ng/ml	0.51 ± 0.17	1.15 ± 0.38	3.27 ± 1.08	MB ACCUBIND ELISA
	0.52 ± 0.17	1.17 ± 0.39	3.17 ± 1.05	MB ACCULITE CLIA
T4 in µg/dl	2.90 ± 0.96	7.53 ± 2.48	16.91 ± 5.58	MB ACCUBIND ELISA
	2.90 ± 0.96	8.37 ± 2.76	16.42 ± 5.42	MB ACCULITE CLIA
TSH in µIU/ml	0.97 ± 0.32	6.50 ± 2.14	34.20 ± 11.29	MB ACCUBIND ELISA
	0.88 ± 0.29	6.15 ± 2.03	31.97 ± 10.55	MB ACCULITE CLIA
fT3 in pg/ml	1.58 ± 0.52	3.46 ± 1.14	6.58 ± 2.17	MB ACCUBIND ELISA
	1.62 ± 0.78	3.52 ± 1.16	6.78 ± 2.24	MB ACCULITE CLIA
fT4 in ng/dl	0.36 ± 0.12	1.76 ± 0.58	3.73 ± 1.23	MB ACCUBIND ELISA
	0.38 ± 0.12	1.63 ± 0.54	3.23 ± 1.07	MB ACCULITE CLIA
T3-Uptake in %U	25.49 ± 2.81	33.22 ± 2.94	46.13 ± 2.95	MB ACCUBIND ELISA
	26.63 ± 2.37	34.60 ± 2.43	49 ± 6.93	MB ACCULITE CLIA
Rapid TSH in µIU/ml	0.87 ± 0.29	6.45 ± 2.13	34.26 ± 11.31	MB ACCUBIND ELISA
	0.77 ± 0.25	6.18 ± 2.04	31.20 ± 10.30	MB ACCULITE CLIA
Thyroid VAST				
(TSH) in µIU/ml	0.98 ± 0.32	7.08 ± 2.34	38.39 ± 12.67	MB ACCUBIND ELISA
	0.94 ± 0.34	6.98 ± 2.30	35.98 ± 11.87	MB ACCULITE CLIA
Strep T3 in ng/ml	0.56 ± 0.18	1.29 ± 0.43	2.88 ± 0.95	MB ACCUBIND ELISA
	0.63 ± 0.22	1.24 ± 0.48	2.65 ± 0.88	MB ACCULITE CLIA
Strep T4 in µg/dl	2.90 ± 0.96	9.11 ± 3.01	13.56 ± 4.47	MB ACCUBIND ELISA
	3.08 ± 1.02	9.32 ± 3.08	12.51 ± 4.13	MB ACCULITE CLIA
Free Thyroid VAST				
(TSH) in µIU/ml	0.79 ± 0.26	7.37 ± 2.43	33.82 ± 11.16	MB ACCUBIND ELISA
	0.78 ± 0.26	7.23 ± 2.39	33.38 ± 11.01	MB ACCULITE CLIA
Strept fT3 in pg/ml	1.43 ± 0.47	3.86 ± 1.27	8.24 ± 2.72	MB ACCUBIND ELISA
	1.59 ± 0.52	4.12 ± 1.36	8.26 ± 2.73	MB ACCULITE CLIA
Strept fT4 in ng/dl	0.31 ± 0.10	1.63 ± 0.54	2.82 ± 0.93	MB ACCUBIND ELISA
	0.33 ± 0.17	1.35 ± 0.45	3.03 ± 1	MB ACCULITE CLIA

ANTI-TG & ANTI-TPO CONTROL - POSITIVE & NEGATIVE **PRODUCT CODE: AIT-101**

LOT# AITPN1B0 **EXP: 02-2025**

INTENDED USE

The Anti-Tg and Anti-TPO Controls are intended for in vitro diagnostic use only as an assayed quality control material to monitor the consistency of performance of Anti-Thyroglobulin and Anti-Thyroid Peroxidase procedures. This product is a human-serum based, liquid control, stabilized with preservatives and can be used with all ELISA, RIA, and CLIA methods.

SUMMARY AND EXPLANATION

The use of quality control material to assist in the assessment of precision in the clinical laboratory is an integral part of laboratory practices. Controls that contain two (2) levels of analytes are necessary to ensure precision and accuracy in immunoassay systems.

REAGENTS

Monobind Inc.'s Anti-TG/TPO Controls are intended to be used in the exact manner as patient samples. The control is packaged as 6 vials of 1.0 ml (3 vials of each level). The antibody activities are adjusted to two distinct concentrations [normal (negative) and elevated (positive] in order to monitor the efficacy of the procedure in use. Follow the manufacturer's instructions for patient dilution to dilute the controls using the diluent supplied with the test.

INSTRUCTIONS FOR USE

1) Bring the vials to room temperature before use.
2) Carefully unscrew and remove cap.
3) Open only one set (normal and elevated) at a time. Dilute according to instructions in the procedure used.
4) Store the unused portions at 2-8 °C after each use.

STORAGE, STABILITY AND DISPOSAL

This product will be stable until the expiration date when stored unopened at 2-8 °C. Once the control is opened, any unused material is stable for 30 days when stored tightly capped at 2-8 °C. To avoid contamination, it is recommended labs aliquot required quantities into vials before each use. Any outdated material should be discarded as biohazardous component.

STORAGE	STABILITY	TEMPERATURE
Unopened	Five (5) years	2 – 8°C
Opened	Thirty (30) days	2 – 8°C

EXPECTED RANGE OF VALUES

These controls have been assayed by leading manufacturers of autoimmune test systems. Values are expressed in IU/ml (WHO: 65/93 for TgAb and 66/387 for TPOAb)

Analyte	Positive Controls		Negative Controls		
	Mean	Range	Range		Method
Anti-Tg in IU/ml	1555.71	1042.32-2069.09	<50	-	MB ACCUBIND ELISA
	1600.56	1072.38-2128.74	<50	-	MB ACCULITE CLIA
Anti-TPO in IU/ml	351.69	235.63-467.74	<20	-	MB ACCUBIND ELISA
	357.61	239.60-475.62	<20	-	MB ACCULITE CLIA

Individual laboratory means should fall within the corresponding acceptable range; however laboratory means may vary from the listed values during the life of this control. Therefore, each laboratory should establish its own means and acceptable ranges for the product used, using Monobind's assignment only as guide. A trend log should be maintained for batch to batch consistency of the test. Variations over time and between laboratories may be caused by a) differences in laboratory personnel, b) improper technique, c) instrumentation and reagents, d) improper dilutions from the stated manufacturer's procedure, and/ or e) modifications in the manufacturer's test procedure.

Refer to http://www.monobind.com/site/qc-documents.html for any updated insert information.

WARNING AND PRECAUTIONS

FOR IN VITRO DIAGNOSTIC USE

All products that contain human serum have been found to be non-reactive for HIV 1&2, HIV-Ag, HBsAg, HCV and RPR by FDA required tests. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Edition, 1988, HHS Publication No. (CDC) 88-8395.

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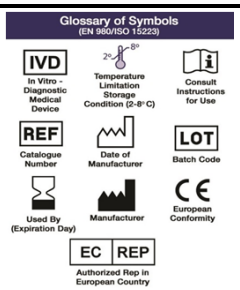

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