Monobind Inc.
Lake Forest, CA 92630, USA

AccuBind
ELISA Microwells

D-Dimer Test System
Product Code: 12025-300

1.0 INTRODUCTION

Intended Use: The Quantitative Determination of D-Dimer Concentration in Human Plasma and Serum by a Microplate Enzyme Immunoassay, Colorimetric

2.0 SUMMARY AND EXPLANATION OF THE TEST

D-Dimer is the term for the cross-linked dimer of fibrinogen degradation product (FDP) D. After fibrinogen is formed in a blood clot, it is broken down through a series of steps so that it can be cleared from the body. D-Dimer is the endpoint of this process which makes its elevation a useful marker for activation of the coagulation and fibrinolytic systems.[1]
D-Dimer concentration may be determined by a blood test to help diagnose thrombosis. Since its introduction in the 1990s, it has become an important test performed in patients with suspected thrombotic disorders. While a negative result practically rules out thrombosis, a positive result may indicate thrombosis but does not definitively rule out other potential causes.[1] Its main use, therefore, is to exclude thromboembolic disease where the probability is low. Additionally, the D-Dimer test can be used in the diagnosis of disseminated intravascular coagulation.[1]
In general circumstances, a D-Dimer value under 500 ng/ml fibrin equivalence units (FEU) excludes deep vein thrombosis (DVT), pulmonary embolism (PE) and other venous thromboembolism (VTE). However, baseline D-Dimer levels increase with age and during pregnancy so modified cut-off values should be implemented for these types of patients to minimize false-positive results.[2-3]
Monitoring D-Dimer levels has become increasingly important as a positive result is indicative of an increased mortality risk. Specifically, detecting high levels of D-Dimer in cancer and pulmonary infection patients is important. VTE is the second highest cause of death in patients with cancer while significant increases in D-Dimer have been linked to higher mortality in those suffering from lung diseases such as COVID-19.[4-6]
The D-Dimer AccuBind® Test System is a quantitative test designed to be sensitive across a wide range of D-Dimer values. The reagents utilize monoclonal mouse antibodies to create a sandwich complex via a simple, fast, and user-friendly protocol.

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.

Upon mixing assay buffer and a serum containing the native antigen, reaction results between the native antigen and the coated antibody, forming an antibody-antigen complex. This interaction is illustrated below:

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Location: Moldova

$$Ag + Ab_{(C)} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} AgAb_{(C)}$$

$Ab_{(C)}$ = Coated Antibody (Excess Quantity)
Ag = Native Antigen (Variable Quantity)
$AgAb_{(c)}$ = Antigen-Antibody complex (Variable Quant.)
k_a = Rate Constant of Association
k_{-a} = Rate Constant of Disassociation

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-coated-antibody sandwich complex on the surface of the wells.

$$AgAb_{(C)} + Ab_{(Enz)} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} Ab_{(Enz)}AgAb_{(C)}$$

$AgAb_{(c)}$ = Antigen-Antibody complex (Variable Quant.)
$Ab_{(Enz)}$ = Enzyme-labeled Antibody (Excess Quant.)
$Ab_{(Enz)}AgAb_{(C)}$ = Sandwich complex (Variable Quant.)
k_a = Rate Constant of Association
k_{-a} = Rate Constant of Disassociation

After another incubation period, the excess enzyme-labeled antibody is separated by washing. The remaining complex is then quantified by addition of substrate that reacts with bound enzyme. The amount of antigen is directly related to the amount of substrate converted by the enzyme.

4.0 REAGENTS

Materials Provided:

A. D-Dimer Calibrators – 1 ml/vial - Icons A-F
Six (6) vials of references for D-Dimer Antigen at levels of 0(**A**), 100(**B**), 400(**C**), 1500(**D**), 4000(**E**) and 10000(**F**) ng/ml FEU. Store at 2-8°C. A preservative has been added.

B. D-Dimer Control – 1 ml/vial – Icon M
One (1) vial of reference control for D-Dimer. Store at 2-8°C. A preservative has been added.

C. Assay Buffer – 12 ml/vial - Icon Ⓑ
One (1) vial containing buffer, dye, and preservatives. Store at 2-8°C.

D. D-Dimer Enzyme Reagent – 13 ml/vial - Icon Ⓔ
One (1) vial containing Enzyme (HRP) labeled Anti-D-Dimer monoclonal mouse IgG in buffer, dye, and preservative. Store at 2-8°C.

E. D-Dimer Antibody Coated Plate – 96 wells - Icon
One 96-well microplate coated with Anti-D-Dimer monoclonal mouse IgG and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

F. 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.

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

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

I. Stop Solution – 8 ml/vial - Icon STOP
One (1) vial containing a strong acid (1N HCl). Store at 2-30°C.

J. 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 plasma (EDTA, Li-Heparin, or Citrate may be used as anticoagulant) or serum in type and the usual precautions in the collection of venipuncture samples should be observed. In order to avoid erroneous results, blood samples should be centrifuged within 15 minutes of collection and the plasma or serum should be removed from the red cells immediately.

Plasma samples may be refrigerated at 2-8°C for a maximum period of three (3) days. Serum samples may be refrigerated at 2-8°C for up to fourteen (14) 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 solution 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 Substrate 'A' into the clear vial labeled Substrate '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. (Duplicate is recommended) **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 Assay Buffer to all wells.
4. Swirl the microplate gently for 20-30 seconds to mix and cover.
5. Incubate 20 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 D-Dimer-Enzyme Reagent to all wells.
   DO NOT SHAKE THE PLATE AFTER ENZYME ADDITION
9. Incubate 20 minutes at room temperature.
10. Wash the wells three (3) times by following steps 6 and 7 as above.
11. 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
12. Incubate at room temperature for fifteen (15) 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 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 D-Dimer 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 D-Dimer 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 D-Dimer 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 (1.761) intersects the dose response curve at (3315 ng/ml) D-Dimer 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.

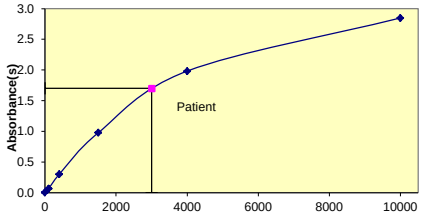
EXAMPLE 1

Sample I.D.	Well Number	Abs (A)	Mean Abs (B)	Value (ng/ml)
Cal A	A1	0.005	0.004	0
	B1	0.003		
Cal B	C1	0.066	0.065	100
	D1	0.065		
Cal C	E1	0.290	0.299	400
	F1	0.308		
Cal D	G1	0.968	0.977	1500
	H1	0.986		
Cal E	A2	2.027	1.982	4000
	B2	1.938		
Cal F	C2	2.881	2.848	10000

	D2	2.815		
Ctrl 1	E2	0.094	0.098	**146**
	F2	0.101		
Ctrl 2	G2	0.951	0.965	**1476**
	H2	0.979		
Patient	A3	1.704	1.761	**3315**
	B3	1.807		

*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 D-Dimer concentrations above 10,000 ng/ml may be diluted 1:10 with the "0" calibrator matrix or other normal serum containing low levels of D-Dimer (< 500 ng/ml) and re-assayed. The sample's concentration is obtained by multiplying the result by the dilution factor and adding the D-Dimer concentration of the diluent used.
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. **A D-Dimer 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

The expected D-Dimer levels for exclusion of thrombosis in plasma samples were obtained from published literature.[2-3, 7] There is general consensus for the following data.

Table 1: Expected Plasma D-Dimer Levels

Patient Age	D-Dimer Level to Exclude Thrombosis
<50 years	<500 ng/ml FEU
>50 years	<Age x 10 ng/ml FEU

To obtain a reference range for serum samples, D-Dimer levels were measured by the D-Dimer AccuBind® Test System in apparently normal adults of different age groups. The values obtained are shown in Table 2.

Table 2: Serum D-Dimer Ranges

Patient Age (years)	N	Average (ng/ml FEU)	Highest (ng/ml FEU)	Lowest (ng/ml FEU)
<50	23	326	1244	137
50-59	19	486	1375	161
60-69	11	467	859	180
70-79	3	440	808	230
80+	3	878	1206	311

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 CHARACTERISITICS

14.1 Precision

The intra-assay precision of the D-Dimer AccuBind® ELISA test system was determined by measuring sixteen (16) replicates of three levels of patient control pools on the same assay run. The results are shown in Table 3.

Table 3: Intra-assay Precision

Sample	N	Mean (ng/ml)	σ	CV%
Control 1	16	200	10.2	5.1
Control 2	16	1934	51.5	2.7
Control 3	16	4237	159.8	3.8

The inter-assay precision (total precision) of the D-Dimer AccuBind® ELISA test system was determined by measuring three levels of patient control pools on three different kits throughout the course of two months. The results are given in Table 4.

Table 4: Inter-assay Precision

Sample	N	Mean (ng/ml)	σ	CV%
Control 1	24	147	9.3	6.4
Control 2	24	1492	96.3	6.5
Control 3	24	3352	174.0	5.2

14.2 Sensitivity

The D-Dimer AccuBind® ELISA test system has a sensitivity of 4.76 ng/ml. The sensitivity was ascertained by determining the variability of the 0 ng/ml calibrator and using the 2σ (95% certainty) statistics to calculate the minimum dose.

14.3 Accuracy

14.3.1 Linearity

The linearity of the D-Dimer AccuBind® ELISA Test System was tested by serially diluting several human plasma and serum samples containing high levels of D-Dimer (up to 11,000 ng/ml) with the "0 ng/ml" serum reference. The observed values were plotted against the expected values and the test system was determined to have excellent linearity up to 11,000 ng/ml with a slope of 0.977 and a correlation factor (R^2) of 0.998.

14.3.2 Recovery

Several human plasma and serum samples containing low levels of D-Dimer (100-700 ng/ml) were spiked with 100, 400, 1200, 4000, and 8000 ng/ml of D-Dimer and assayed on the D-Dimer AccuBind® ELISA Test System. The system demonstrated excellent recovery with all observed values falling within 15% of the expected values.

14.4 Specificity

The following substances were tested on the D-Dimer AccuBind® ELISA test system to determine interference and cross-reactivity. The results are tabulated below.

Substance	Cross Reactivity	Concentration
D-Dimer	1.0000	----
Fibrogen	< 0.0001	4mg/ml
Plasminogen	< 0.0001	150ng/ml
Angiostatin	< 0.0001	150ng/ml
tPA	< 0.0001	150ng/ml
PAI1	< 0.0001	150ng/ml

15.0 REFERENCES

1. Johnson ED, Schell JC, Rodgers GM. The D-Dimer assay. *American Journal of Hematology.* **94**: 833-839. 2019. doi:10.1002/ajh.25482
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Revision: 1
Date: 2021-MAR-30 DCO: 1479
MP12025 Product Code: 12025-300

Size		96(A)	192(B)
Reagent (fill)	A)	1ml set	1ml set
	B)	1ml	1ml
	C)	12ml	2 (12ml)
	D)	13ml	2 (13ml)
	E)	1 plate	2 plates
	F)	1 (20ml)	1 (20ml)
	G)	1 (7ml)	2 (7ml)
	H)	1 (7ml)	2 (7ml)
	I)	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

25-OH Vitamin D Total (Vit D-Direct) Test System

Product Code: 9425-300

1.0 INTRODUCTION

Intended Use: The Quantitative Determination of 25-OH Vitamin D Concentration in Human Serum by a Microplate Enzyme Immunoassay, Colorimetric

2.0 SUMMARY AND EXPLANATION OF THE TEST

Vitamin D is a fat soluble secosteroid hormone that is important in the management of calcium and phosphorus concentrations required in the mineralization of bone. Vitamin D has two important forms: cholecalciferol (D_3) formed in the skin from ultraviolet light and ergocalciferol (D_2) found in dairy products. However, these forms do not have significant biological activity. The hormonal active form, 1, 25-dihydroxylcholecalciferol, is produced through transformations in the liver and kidney. The first step in this conversion is an enzymatic reaction of D_2 or D_3 into 25OH-D_2 or 25OH-D_3. These 25OH D forms are not freely circulating in blood, but are primarily bound to vitamin D binding protein (VDBP). The high binding affinity of the 25OH $D_{(2 \text{ or } 3)}$ compared to other derivatives of vitamin D leads to a long half-life in blood and its use as an accurate indicator of Vitamin D status. Vitamin D deficiency has been associated to diseases related to bone damage such as osteomalacia and rickets. Vitamin D can be dietarily supplemented through the use of Vitamin D_2 or vitamin D_3. The sum of the 25OH $D_{(2 \text{ and } 3)}$ in serum or plasma is referred to as total 25OH Vitamin D. The accurate measurement of total vitamin D is necessary in monitoring deficient vitamin D patients to achieve the optimum dosage and avoid excessive levels, which are considered toxic.

3.0 PRINCIPLE

Sequential Competitive Method (Type 6):
The essential reagents required for a solid phase sequential enzyme immunoassay include immobilized antibody, enzyme-antigen conjugate and native antigen. Upon mixing immobilized antibody, and a serum sample containing the native antigen, a binding reaction results between the native antigen for a limited number of insolubilized binding sites. The interaction is illustrated by the following equation:

$$Ag + Ab_{CW} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} AgAb_{CW}$$

Ab_{CW} = Monospecific Immobilized Antibody (Constant Quantity)
Ag = Native Antigen (Variable Quantity)
$AgAb_{CW}$ = Antigen-Antibody Complex
k_a = Rate Constant of Association
k_{-a} = Rate Constant of Disassociation
$K = k_a / k_{-a}$ = Equilibrium Constant

After removing any unreacted native antigen by a wash step, the enzyme-conjugated antigen is introduced. The conjugate reacts with sites of the antibody unoccupied by the native antigen.

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

^{Enz}Ag = Enzyme-antigen Conjugate (Constant Quantity)
$^{Enz}AgAb_{CW}$ = Enzyme-antigen Conjugate-Antibody Complex

After a short second incubation, the antibody-bound fraction is separated from unbound antigen by decantation or aspiration. The enzyme activity in the antibody-bound fraction is inversely proportional to the native antigen concentration. By utilizing several different calibrators 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. Vit D Calibrators – 1ml/vial – Icons A-G
Seven (7) vials containing human serum albumin reference calibrators for 25-OH Vitamin D at concentrations of 0 (**A**), 5 (**B**), 10 (**C**), 25 (**D**), 46 (**E**), 85 (**F**), and 150 (**G**) in ng/ml. A preservative has been added. Store at 2-8°C.
The calibrators can be expressed in molar concentrations (nM/L) by multiplying by 2.5. For example:
10ng/ml x 2.5 = 25nM/L

B. Vit D Controls – 1ml/vial – Icons M-N
Two (2) vials containing human serum reference controls at concentration established (exact value listed on label). A preservative has been added. Store at 2-8°C.

C. Vit D Releasing Agent – 12 ml/vial – Icon
One (1) vial containing vitamin D binding protein releasing agents. Store at 2-8°C.

D. Vit D Enzyme Reagent – 12 ml/vial – Icon
One (1) vial containing 25-OH Vitamin D_3 (Analog)-horseradish peroxides (HRP) conjugate in a protein-stabilizing matrix. Store at 2-8°C.

E. Vit D Antibody Coated Plate – 96 wells – Icon
One 96-well microplate coated with < 1.0 µg/ml anti-Vitamin D sheep IgG and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

F. 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.

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

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

I. Product Insert

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 & 0.100ml (25 & 100µl) with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.050, 0.100 & 0.350ml (50, 100 & 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. 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 requirements.

6.0 SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood, serum 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) with no anti-coagulants. Allow the blood to clot for serum samples. Centrifuge the specimen to separate the serum from the cells.

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 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, 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 extracted 25-OH Vitamin D calibrator, control or specimen into the assigned well.
3. Add 0.100 ml (100 µl) of the 25-OH Vitamin D Releasing Agent to all wells.
4. Mix **(Note 3)** the microplate for 20-30 seconds until homogeneous.
5. Cover and incubate for 30 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 0.350 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 25-OH Vitamin D Enzyme Reagent to all wells.

DO NOT SHAKE THE PLATE AFTER ADDITION

9. Cover and incubate for 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 0.350 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.**
12. 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 (MIX) THE PLATE AFTER SUBSTRATE ADDITION

13. Incubate at room temperature for twenty (20) minutes.
14. Add 0.050 ml (50 µl) 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.**
15. 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.**

Note1: **Do not use the substrate reagent if it looks blue.**
Note 2: **Do not use reagents that are contaminated or have bacteria 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 4: **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 25-OH Vitamin D 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 25-OH Vitamin D concentration in ng/ml on linear graph paper (do not average the duplicates of the calibrators before plotting).
3. Connect the points with a best-fit curve.
4. To determine the concentration of 25-OH Vitamin D 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 (1.033) intersects the dose response curve at 39.9 ng/ml 25-OH Vitamin D concentration (See Figure 1).

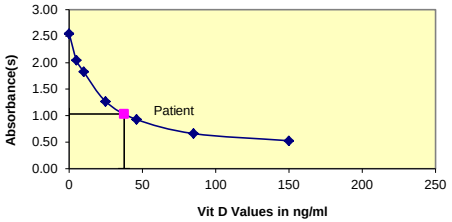
Note: Computer data reduction software designed for ELISA assay may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained.

EXAMPLE 1

Sample I.D.	Well Number	Abs (A)	Mean Abs (B)	Value (ng/ml)
Cal A	A1	2.559	2.548	0
	B1	2.537		
Cal B	C1	2.041	2.047	5
	D1	2.054		
Cal C	E1	1.848	1.826	10
	F1	1.804		
Cal D	G1	1.286	1.267	25
	H1	1.249		
Cal E	A2	0.934	0.930	46
	B2	0.927		
Cal F	C2	0.654	0.663	85
	D2	0.712		
Cal G	G2	0.511	0.529	150
	H2	0.546		
Pat# 1	A3	1.027	1.033	37.5
	A4	1.039		

*The above data and figure below is for example only. Do not use utilize it for calculating results.

Figure 1



Note: Multiply the horizontal values by 2.5 to convert into nM/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 A (0 ng/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, is 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 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.

13.0 EXPECTED RANGES OF VALUES

Based on the published literature the following ranges have been assigned. **These ranges should be used as guidelines only:**

TABLE 1
Expected Values for the Vit D-Direct ELISA

LEVEL	RANGE (ng/ml)
Very severe vitamin D deficiency	< 5
Severe vitamin D deficiency	5-10
Vitamin D deficiency	10-20
Suboptimal vitamin D provision	20-30
Optimal vitamin D level	30-50
Upper norm	50-70
Overdose, but not toxic	70-150
Vitamin D intoxication	> 150

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 25-OH Vitamin D 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
Within Assay Precision

Serum	N	X	σ	%C.V.
1	20	22.16	1.35	6.10
2	20	34.96	1.44	4.11
3	20	86.09	6.37	7.40

TABLE 3
Between Assay Precision

Serum	N	X	σ	%C.V.
1	45	23.88	2.14	8.96
2	45	37.53	3.44	9.17
3	45	87.91	7.1	8.08

14.2 Sensitivity

The sensitivity of the Vit-D Direct AccuBind® ELISA test system method was ascertained by determining the variability of the '0' calibrator and using the 2σ (95% certainty) statistic to calculate the minimum dose. The test system has an analytical sensitivity of 1.14 ng/ml Vitamin D.

14.3 Accuracy

The Vit D AccuBind® ELISA Test System was compared with a reference method. A total of 83 biological specimens from low, normal, and high Vit D level populations were used; the values ranged from 9.5ng/ml to 200ng/ml. The least square regression equation and the correlation coefficient were computed for the AccuBind method when compared to the reference method. The data obtained is displayed in Table 4.

Method	Mean	Leas Square Regression Analysis	Correlation Coefficient
Monobind (y)	52.08	y=1.02(x)+1.33	0.918
Reference (x)	49.98		

14.4 Specificity

The % cross-reactivity of the 25-OH Vitamin D 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 25-OH Vitamin D needed to displace the same amount of labeled analog.

TABLE 5

Substance	Cross Reactivity
25-OH Vitamin D2	1.0000
25-OH Vitamin D3	1.0000
Vitamin D2	0.0076
Vitamin D3	0.0039
D2 Active 1,3,25-Hydroxy Vitamin D 2	1.9000
D3 Active 1,3,25-Hydroxy Vitamin D 3	1.1500

15.0 REFERENCES

1. Holick, MF. "Vitamin D Status: Measurement, Interpretation and Clinical Application". *Ann Epidemoil*. 2009, 19(2):73 - 78
2. Morris H. A. "Vitamin D: A Hormone for All Seasons-How Much is enough?" *Clin. Biochem*. Rev., 2005, 26, 21-32.
3. Bikle D. D. "Vitamin D and the skin". *J. Bone Miner. Metab.*, 2010, 28, 117-30.
4. Zerwekh J. E. "Blood biomarkers of vitamin D status". *Am. J. Clin. Nutr*. 2008, 87, 1087S-91S.
5. Moyad M. A. "Vitamin D: a rapid review". *Dermatol Nurs.*, 2009, 21, 25-30.

Effective Date: 2018-Jan-10 Rev. 2 **DCO: 1275**
MP9425 **Product Code: 9425-300**

Size		96(A)	192(B)	480(D)	960(E)
Reagent (fill)	A)	1ml set	1ml set	2ml set	2(2ml set)
	B)	1ml set	1ml set	2ml set	2(2ml set)
	C)	1 (12ml)	2 (12ml)	1 (60ml)	2 (60ml)
	D)	1 (12ml)	2 (12ml)	1 (60ml)	2 (60ml)
	E)	1 plate	2 plate	5 plate	10 plate
	F)	1 (20ml)	1 (20ml)	1 (60ml)	2 (60ml)
	G)	1 (12ml)	2 (12ml)	1 (52ml)	2 (52ml)
	H)	1 (8ml)	2 (8ml)	1 (30ml)	2 (30ml)

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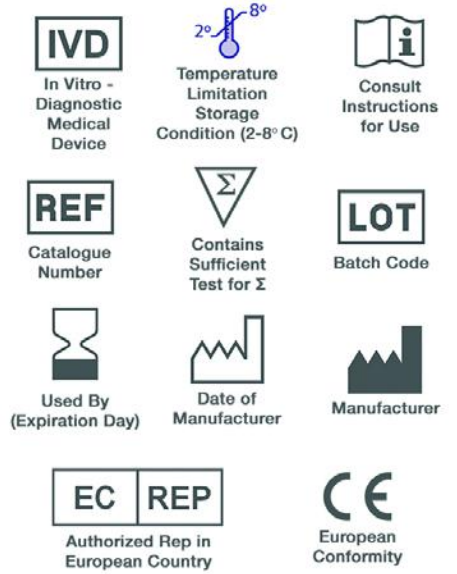
IVD 2° 8° CE
EC REP CEpartner4U, Esdoornlaan 13 3951 DBMaarn, The Neatherlands www.cepartner4u.eu

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

Glossary of Symbols
(EN 980/ISO 15223)



Monobind Inc.
Lake Forest, CA 92630, USA

AccuBind
ELISA Microwells

Vitamin B12 (Vit B12) Test System
Product Code: 7625-300

1.0 INTRODUCTION

Intended Use: The Quantitative Determination of Vitamin B12 Concentration in Human Serum by a Microplate Enzyme Immunoassay, Colorimetric

2.0 SUMMARY AND EXPLANATION OF THE TEST

Vitamin B12 is one of the nine water soluble vitamins important for healthy body functioning. The most important roles Vitamin B12 plays in the human body are in the formation of red blood cells and the formation of the myelin sheath around the nerves. Since the effects are seen in body systems with a large range of function, the symptoms of Vitamin B12 deficiency can sometimes be very ambiguous. A deficiency may also take from months to years to manifest depending on the cause and severity.[1,2,3]

Two of the most common causes of Vitamin B12 deficiency are diet and age. Because most sources of dietary Vitamin B12 come from animals, vegans who do not efficiently supplement their diet are at risk. The elderly community is also at high risk because of their diet, as well as the less efficient functioning of their digestive system.[1,3,4]

Intake of Vitamin B12 starts by ingestion and then digestion by saliva. Once reaching the gut, Vitamin B12 bound to proteins in food are released by the acids present. The B12 can then bind the Intrinsic factor. Once bound to IF, Vitamin B12 is stable enough to travel into the intestines where it can be absorbed into your body through of its association with IF.[1,5,6,7]

Two very useful tests to distinguish between Vitamin B12 deficiency and folate deficiency are methylmalonyl CoA (MMA) and homocysteine (hcy). Both deficiencies are represented by similar symptoms; however, even though both show increased levels of homocysteine, only Vitamin B12 deficiency causes an increase in methylmalonyl CoA. The increase in levels of methylmalonyl CoA and homocysteine is thought to be the root cause of any symptoms that accompany a Vitamin B12 deficiency. High levels of these two analytes in the blood stream causes increased oxidative stress to cells therefore causing increased apoptosis. In turn, vascular disease results in the form of atherosclerosis, coronary heart disease and/or neurodegeneration (ex. Parkinson's Disease).[1,8,9]

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.

$AgAb_{Btn} + {}^{Enz}AgAb_{Btn} + \underline{Streptavidin}_{CW} \Rightarrow \underline{immobilized\ complex}$

$\underline{Streptavidin}_{CW}$ = 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. Vitamin B12 Calibrators – 1ml/vial - Icons A-F
Six (6) vials containing human serum albumin reference calibrators for Vitamin B12 at concentrations of 0 (**A**), 100 (**B**), 200 (**C**), 400 (**D**), 1000 (**E**), and 2000 (**F**) in pg/ml. A preservative has been added. Store at 2-8°C.
The calibrators can be expressed in molar concentrations (pM/L) by multiplying by 0.738. For example:
100pg/ml x 0.738= 73.8 pM/L

B. Vitamin B12 Enzyme Reagent – 7.0 ml/vial – Icon Ⓔ
One (1) vial containing Vitamin B12 (Analog)-horseradish peroxides (HRP) conjugate in a protein-stabilizing matrix. Store at 2-8°C.

C. Vitamin B12 Biotin Reagent – 7.0 ml/vial – Icon ∇
One (1) vial containing anti-Vitamin B12 biotinylated purified rabbit IgG 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 – 20.0 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 – 12.0 ml/vial – Icon S^N
One (1) vial containing tetramethylbenzidine (TMB) and hydrogen peroxide (H_2O_2) in buffer. Store at 2-8°C.

G. Stop Solution – 8.0 ml/vial – Icon STOP
One (1) vial containing a strong acid (H_2SO_4). Store at 2-8°C

H. Releasing Agent – 14.0 ml/vial – Icon Ξ
One (1) vial containing a strong base (sodium hydroxide) and potassium cyanide. Store at 2-8°C.

I. Stabilizing Agent – 0.7 ml/vial – Icon Π
One (1) vial containing tris 2-carboxyethyl)phosphine (TCEP) solution. Store at 2-8°C.

J. Neutralizing Buffer – 7.0 ml/vial – Icon NZ
One (1) vial containing buffer with dye that reduces the pH of sample extraction. Store at 2-8°C.

K. Product Insert

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 0.050 & 0.100ml (50 & 100µl) 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. Adjustable volume (200-1000µl) dispenser(s) for conjugate.
4. Glass test tubes for calibrators, control, and patient sample preparation.
5. Microplate washer or a squeeze bottle (optional).
6. Microplate Reader with 450nm and 620nm wavelength absorbance capability.
7. Absorbent Paper for blotting the microplate wells.
8. Plastic wrap or microplate cover for incubation steps.
9. Vacuum aspirator (optional) for wash steps.
10. Timer.
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 labor-atory 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 requirements.

6.0 SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood, serum 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) with no anti-coagulants. Allow the blood to clot for serum 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, 0.100ml (100µl) 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. EXTRACTION AGENT
Add an aliquot of the stabilizing agent in order to prepare a 1/40 (stabilizing agent / releasing agent) dilute solution. For example, to make 4ml (4000µl), add 0.100ml (100µl) stabilizing agent to 3.9ml (3900µl) releasing agent.

3. SAMPLE EXTRACTION (*See Note 3)*
Obtain enough test tubes for preparation of all patient samples, controls, and calibrators. Dispense 0.10ml (100µl) of all samples into individual test tubes. Pipette 0.050ml (50µl) of the prepared extraction agent to each test tube, shaking *(see note 3)* after each addition. Let the reaction proceed for 15 min. At end of the 15 min, dispense 0.050 ml (50µl) of the neutralizing buffer, vortex *(see note 3)*.

Note1: Do not use the working substrate if it looks blue.
Note 2: Do not use reagents that are contaminated or have bacteria growth.
Note 3: Use of multiple (3) touch vortex is recommended.
Note 4: It is extremely important to accurately dispense the correct volume with a calibrated pipette and by adding near the bottom of the glass tubes at an angle while touching the side of the tubes.
Note 5: Samples with high protein concentration should be diluted 1:1 with a saline solution before performing the extraction.
Note 6: See www.monobind.com/education-center for Step-by-Step Guide on Sample Extraction for Vitamin B12 (& Folate) in Lab Tips

9.0 TEST PROCEDURE

Before proceeding with the assay, bring all reagents, 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 extracted Vitamin B12 calibrator, control or specimen into the assigned well.
3. Add 0.050 ml (50 µl) of the Vitamin B12 Biotin Reagent to all wells.
4. Mix the microplate gently for 20-30 seconds to mix.
5. Cover and incubate for 45 minutes at room temperature.
6. Add 0.050 ml (50 µl) of Vitamin B12 Enzyme Reagent to all wells.
   Add directly on top the reagents dispensed in the wells.
7. Mix the microplate gently for 20-30 seconds to mix.
8. Cover and incubate for 30 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 0.350 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.**
11. 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 THE PLATE AFTER SUBSTRATE ADDITION
12. Incubate at room temperature for twenty (20) minutes.
13. Add 0.050 ml (50 µl) 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 2000pg/ml 1:5 and 1:10 with Vitamin B12 '0' pg/ml calibrator and re-assay.

10.0 CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of Vitamin B12 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 Vitamin B12 concentration in pg/ml on linear graph paper (do not average the duplicates of the calibrators before plotting).
3. Connect the points with a best-fit curve.
4. To determine the concentration of Vitamin B12 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 (1.53) intersects the dose response curve at 391.4 pg/ml Vitamin B12 concentration (See Figure 1).

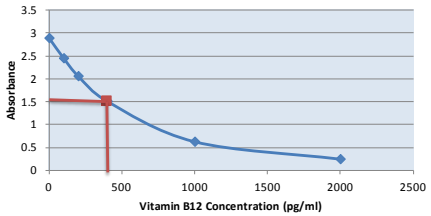
Note: Computer data reduction software designed for ELISA assay may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained.

EXAMPLE 1

Sample I.D.	Well Number	Abs (A)	Mean Abs (B)	Value (pg/ml)
Cal A	A1	2.898	2.89	0
	B1	2.891		
Cal B	C1	2.495	2.45	100
	D1	2.415		
Cal C	E1	2.107	2.06	200
	F1	2.023		
Cal D	G1	1.544	1.51	400
	H1	1.468		
Cal E	A2	0.662	0.63	1000
	B2	0.604		
Cal F	C2	0.263	0.25	2000
	D2	0.239		
Pat# 1	G2	1.479	1.53	391.4
	H2	1.573		

*The above data and table below is for example only. Do not use it for calculating your results.

Figure 1: Vitamin B12



Note: Multiply the horizontal values by 0.738 to convert into pM/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, 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, is 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 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.

13.0 EXPECTED RANGES OF VALUES

In agreement with established reference intervals for a "normal" population the expected ranges for the Vitamin B12 AccuBind® ELISA Test System are detailed in Table 1.

TABLE 1
Expected Values - Vit B12 AccuBind® ELISA Test System[12]

Population	pg/ml	pmol/L
Newborn	160 - 1300	118-959
Adult	200 – 835	148 – 616
Adult > 60 y	110 – 800	81 – 590

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 Vitamin B12 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 pg/ml)

Sample	N	X	σ	C.V.
Low	20	334.8	24.3	7.3%
Normal	20	484.9	17.6	3.6%
High	20	925.3	28.3	3.1%

TABLE 3
Between Assay Precision (Values in pg/ml)

Sample	N	X	σ	C.V.
Low	18	314.9	49.4	15.7%
Normal	18	441.3	46.7	10.6%
High	18	913.1	39.4	4.8%

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

14.2 Sensitivity

The Vitamin B12 AccuBind® ELISA Test System has a sensitivity of 70.13 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 Vitamin B12 AccuBind® ELISA Test System was compared with a reference method. Biological specimens from low, normal and relatively high Vitamin B12 level populations were used (the values ranged from 156 pg/ml – 1830 pg/ml). The total number of such specimens was 56. The least square regression equation and the correlation coefficient were computed for this Vitamin B12 test 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
This Method (Y)	654.3	y= 1.0186x -48.82	0.9506
Reference (X)	690.2		

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 Vitamin B12 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 Vitamin B12 needed to displace the same amount of labeled analog.

TABLE 5

Substance	Cross Reactivity
Bilirubin	0.0003
Rhematoid Factor	0.0008
Cobinamide	<0.0001
Lipemia	<0.0001
Hemoglobin	<0.0001

15.0 REFERENCES

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Effective Date: 2019-Jul-16 Rev. 6 **DCO: 1353**
MP7625 **Product Code: 7625-300**

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 (12ml)	2 (12ml)
	G)	1 (8ml)	1 (8ml)
	H)	1 (14ml)	2 (14ml)
	I)	1 (0.7ml)	2 (0.7ml)
	J)	1 (7ml)	2 (7ml)

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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

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[2,6] 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.

$AgAb_{Btn} + {}^{Enz}AgAb_{Btn} + \underline{Streptavidin}_{CW} \Rightarrow \underline{immobilized\ complex}$

$\underline{Streptavidin}_{CW}$ = 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).

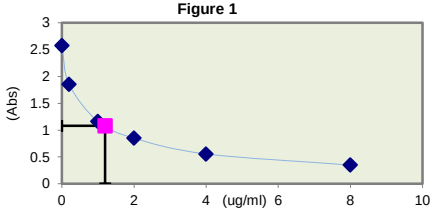
Note: Computer data reduction software designed for ELISA assays may also be used for the data reduction. If such

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

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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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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.

$AgAb_{Btn} + {}^{Enz}AgAb_{Btn} + \underline{Streptavidin}_{CW} \Rightarrow \underline{immobilized\ complex}$

$\underline{Streptavidin}_{CW}$ = Streptavidin immobilized on well
$\underline{Immobilized\ complex}$ = 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. 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).

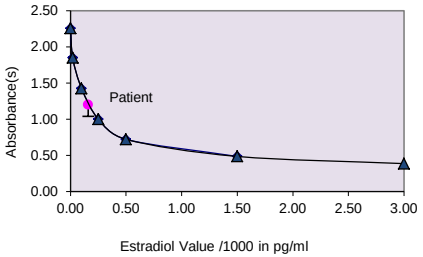
Note: Computer data reduction software designed for ELISA assay may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained.

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 $\geq$ 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

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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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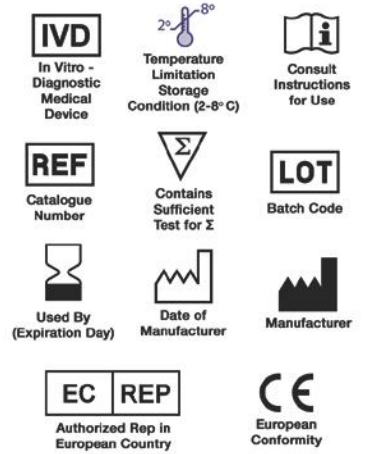
Glossary of Symbols
(EN 980/ISO 15223)



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).

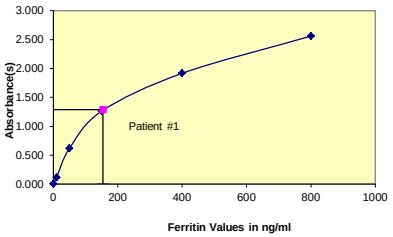
Note: Computer data reduction software designed for ELISA assays may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained.

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 2°-8° CE
EC REP CEpartner4U, Esdoornlaan 13 3951 DB Maarn, The Nasthorlands 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

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 -

DIA.PRO
Diagnostic Bioprobes Srl
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(Milano) - Italy
Phone +39 02 27007161
Fax +39 02 44386771
e-mail: info@diapro.it

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:
Dia.Pro Diagnostic Bioprobes S.r.l.
Via G. Carducci n° 27 – Sesto San Giovanni (MI) – Italy

CE
0318

HBsAg

Confirmation

Set of Reagents for the confirmation of HBsAg positivity in human sera or plasma

- for “in vitro” diagnostic use only -

DIA.PRO
Diagnostic Bioprobes Srl
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Code SCONF.CE
20/40 tests

HBsAg Confirmation

A. INTENDED USE

In the screening of blood units for Hepatitis B surface Antigen or HBsAg some false positivity may happen, leading to a misinterpretation of the assay results and a misclassification of the blood unit and the donor.
To confirm the positivity of a screened sample or to confirm the presence of an ongoing HBV infection in a hospitalized patient, a confirmatory test has to be run.
A simple procedure based on an immunoreaction of neutralization is used in combination with the HBsAg assay.

B. PRINCIPLE OF THE ASSAY

The device has to be used in combination with the products code SAG1.CE/SAG1ULTRA.CE for the determination of HBsAg in human sera and plasma.
The sample, whose repeatedly positivity for HBsAg has to be confirmed, is premixed with a reagent containing high titer anti HBsAg antibodies that will neutralize the antigen is really present in the sample.
The neutralized sample is then tested for HBsAg according to the procedure reported for the specific device.
If the positivity in the first screening test is specifically related to the presence of HBsAg in the sample, the same will not react any more in the assay having been neutralized by the antibody.
If at contrary the positivity of the sample is not abolished by the neutralization reaction, this reactivity is not due specifically to the presence of HBsAg in the sample, but to some interfering substance.

C. CONTENT OF THE KIT

The set contains the following reagents**.**

1. Neutralizing Reagent SOLN NEUT
It contains high titer human plasma positive for anti HBsAg antibodies, 0.2 mg/ml gentamicine sulphate and 0.1% Kathon GC as preservatives.

2. Control Reagent CONTROL
It contains human plasma negative for anti HBsAg antibodies, 0.2 mg/ml gentamicine sulphate and 0.1% Kathon GC as preservatives.

3. Assay Diluent DILSPE
0.15 M NaCl phosphate buffered solution pH 7.0 ± 0.2 containing 0.1% Kathon GC for the dilution of over ranging samples .

Note: *Reagents have been tested and found negative for HBsAg, HCV Ab and HIV Ab with CE-marked kits.*

Number of tests	**20**	**40**
Code	**SCONF.CE**	**SCONF.CE.40**
Control Reagent (CONTROL)	1x4ml/vial	1x8ml/vial
Neutralizing Reagent (SOLN NEUT)	1x4ml/vial	1x8ml/vial
Phosphate Buffered Saline (DILSPE)	1x30ml/vial	1x60ml/vial
Package insert	N°1	N°1

D. MATERIALS REQUIRED BUT NOT PROVIDED

1. CE marked devices for HBsAg determination:

Product	**Code**	**Tests**
HBsAg one	SAG1.CE	192
	SAG1.CE.96	96
	SAG1.CE.480	480
	SAG1.CE.960	960
HBsAg one version ULTRA	SAG1ULTRA.CE	192
	SAG1ULTRA.CE.96	96
	SAG1ULTRA.CE.480	480
	SAG1ULTRA.CE.960	960

2. Isotonic sterile solution.
3. Calibrated Micropipettes and disposable plastic tips.
4. Timer with 60 minute range or higher.
5. Absorbent paper tissues.
6. Calibrated ELISA microplate thermostatic incubator (dry or wet), capable to provide shaking at 1300 rpm+/-150, set at +37°C.
7. Calibrated ELISA microwell reader with 450nm (reading) and with 620-630nm (blanking) filters.
8. Calibrated ELISA microplate washer.
9. Vortex or similar mixing tools.
10. Disposable plastic tube of 2-5 ml.

E. WARNINGS AND PRECAUTIONS

1. For “in vitro” diagnostic use only.
2. The Set has to be used by skilled and properly trained technical personnel only, under the supervision of a medical doctor responsible of the laboratory.
3. When the device is used for confirmation of a sample repeatedly positive in the screening of blood units and blood components, it has to be used in a laboratory certified and qualified by the national authority in that field (Ministry of Health or similar entity) to carry out this type of analysis.
4. The laboratory environment should be controlled so as to avoid contaminants such as dust or air-born microbial agents, when opening the vials contained in the set.
5. Upon receipt, store the kit at 2..8°C into a tem perature controlled refrigerator or cold room.
6. Do not interchange Reagents between different lots of the device. It is even recommended that Reagents between two sets of the same lot are not interchanged.
7. Check that the Reagents of the device are clear and do not contain visible heavy particles or aggregates. If not, advise the laboratory supervisor to initiate the necessary procedures for kit replacement.
8. Avoid cross-contamination between kit reagents by using disposable tips and changing them between the use of each one.
9. Do not use the set after the expiration date stated on the external container and internal (vials) labels.
10. 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.
11. Waste produced during the use of the set in combination with the devise for HBsAg determination 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 before waste. 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..
12. Accidental spills from samples and operations 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.
13. 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.
14. Refer to the Instructions for Use of the product code SAG1.CE/SAG1ULTRA.CE used in combination for the confirmation assay.

F. SPECIMEN: PREPARATION AND WARNINGS

1. The sample turned out to be repeatedly positive in the first HBsAg determination with HBsAg One has to be used for the test of neutralization. Treat the sample as described in section L.
2. Avoid any addition of preservatives to samples after first screening; 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 (red) and lipemic ("milky") samples have to be discarded by definition anyway as they could generate false results in the test for HBsAg.
5. Samples containing residues of fibrin or heavy particles or microbial filaments and bodies should be discarded as well as they could give rise to false positive results both in HBsAg first assay and even in the confirmation one.
6. The assay is not suitable to confirm the negativity of samples that turned out to be negative in the first HBsAg screening test.
7. Sera and plasma can be stored at +2°..8°C for up t o five days after collection. For longer storage periods, samples can be stored frozen at –20°C for several months.
8. Any frozen sample should not be frozen/thawed more than once as this may generate particles that could affect the test result. If some turbidity is present or presence of micro particles is suspected after thawing, filter the sample on a disposable 0.2-0.8u filter to clean it up for testing or use the two-steps alternative method.
9. Refer to the Instructions for Use of the products code SAG1.CE/SAG1ULTRA.CE used in combination for the confirmation assay.

G. 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 (70% ethanol, 10% solution of bleach, hospital grade disinfectants) of those parts that could accidentally come in contact with the sample or the components of the kit. 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 $\pm$1°C) and regularly checked to ensure the correc t 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. In case of **shaking** during incubations, the instrument has to ensure 350 rpm $\pm$150. Amplitude of shaking is very important as a wrong one could give origin to splashes and therefore to some false positive result.
4. The **ELISA washer** is extremely important to the overall performances of the assay. The washer must be carefully validated and correctly optimized using the kit controls/calibrator and reference panels, before using the kit for routine laboratory tests. Usually 4-5 washing cycles (aspiration + dispensation of 350ul/well of washing solution = 1 cycle) are sufficient to ensure that the assay performs as expected. A soaking time of 20-30 seconds between cycles is suggested. In order to set correctly their number, it is recommended to run an assay with the kit controls/calibrator and well-characterized negative and positive reference samples, and check to match the values reported below in the section "Internal Quality Control". Regular calibration of the volumes delivered and maintenance (decontamination and cleaning of needles) of the washer has to be carried out according to the instructions of the manufacturer.
5. **Incubation times** have a tolerance of $\pm$5%.
6. The **microplate reader** has to be equipped with a reading filter of 450nm and with a second filter (620-630nm) strongly recommended for blanking purposes. Its standard performances should be (a) bandwidth $\leq$ 10 nm; (b) absorbance range from 0 to $\geq$ 2.0; (c) linearity to $\geq$ 2.0; repeatability $\geq$ 1%. 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 that the correct optical density is measured. It should be regularly maintained according to the manufacturer 's instructions.
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 full compliance with the essential requirements of the assay. Support is also provided for the installation of new instruments to be used in combination with the kit.

H. PRE ASSAY CONTROLS AND OPERATIONS

1. Check the expiration date of the set printed on the external label of the kit box. Do not use if expired.
2. Check that the liquid components of the set are not contaminated by naked-eye visible particles or aggregates.
3. Allow components to reach room temperature (about 1 hr) and then mix them on vortex.
4. Check that micropipettes are set to the required volume.
5. Check that equipments and the kits SAG1.CE/SAG1ULTRA.CE used in combination are available and ready to use.
6. In case of problems, do not proceed further with the test and advise the supervisor.
7. Refer to the Instructions for Use of the product code SAG1.CE/SAG1ULTRA.CE used in combination for the confirmation assay.

I. ASSAY PROCEDURE

The confirmation assay reported below has to be carried out on a sample repeatedly positive for HBsAg, when the product code SAG1.CE/SAG1ULTRA.CE is used for first screening.
The test is not suitable to confirm negative samples.
The Negative Control and the Calibrator of the kit code SAG1.CE/SAG1ULTRA.CE have always to be run whenever the assay of confirmation is used.

Samples with OD450nm < 2

If the sample gave an optical signal < 2 OD450nm in the screening test use the following distribution protocol:

1. Add 50 ul Neutralizing Reagent to 150 ul sample to be confirmed in a disposable test tube (**N**). Mix on vortex.
2. Add 50 ul Control Reagent to 150 ul sample to be confirmed in a second disposable test tube (**C**). Mix on vortex.
3. Incubate both tubes for 30 min at room temperature.
4. Then follow the Instructions for Use of the product code SAG1.CE/SAG1ULTRA.CE and determine HBsAg reactivity in both N and C.

Samples with OD450nm > 2

If the sample gave an optical signal > 2.000 OD450nm in the screening test, use the following procedure:

1. Dilute the sample 1:100 by dispensing 5 ul of specimen and 495 ul of Assay Diluent in a disposable tube (S01K). Mix on vortex.
2. Dilute further 1:10,000 the sample by dispensing 5 ul of the 1:100 solution and 495 ul of Assay Diluent in a disposable tube (S10K). Mix on vortex.
3. In a first test tube dispense 150 ul S01K and add 50 ul control Reagent (**C01K**). Mix on vortex.
4. In a second test tube dispense again 150 ul S01K and add 50 ul Neutralizing Reagent (**N01K**). Mix on vortex.
5. In a third test tube dispense 150 ul of solution S10K and add 50 ul Control Reagent (**C10K**). Mix on vortex.
6. In a fourth test tube dispense again 150 ul of solution S01K and add 50 ul Neutralizing Reagent (**N10K**). Mix on vortex.
7. Incubate all these tubes for 30 min at room temperature.
8. Then follow the Instructions for Use of the product code SAG1.CE/SAG1ULTRA.CE and determine HBsAg reactivity in all the tubes (C01K, C10K, N01K and N10K).
9. If the OD450nm value of the 1:10,000 dilution is still greater than 2 for the non-neutralized sample (control well), repeat the test after further diluting the sample 1:100,000.

An example of dispensation scheme is reported in the following table:

Microplate

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	S2 C										
B	NC	S2 N										
C	NC	S3 C										
D	NC	S3 N										
E	CAL C	S4 C										
F	CAL N	S4 N										
G	S1 C	S5 C										
H	S1 N	S5 N										

Legenda : BLK = Blank NC = Negative Control CAL = Calibrator S = Sample C = Control N = Neutralizing Reagent

L. CALCULATION OF RESULTS

The positivity of the specimen is confirmed if the ratio between the OD450nm value for the control well (**C**) and the OD450nm value for the neutralization well (**N**) is higher than 2, that is formulated mathematically as follows:

$$C / N > 2$$

If a HBsAg positive sample shows a ratio C/N < 2 in the neutralization assay it is considered false positive.

M. INTERNAL QUALITY CONTROL

A check is performed any time the kit is used in combination with the device for HBsAg determination (code SAG1.CE/SAG1ULTRA.CE) in order to assure full matching the expected performances.

In particular ensure that the following results are met:

Parameter	Requirements
Blank well	< 0.100 OD450nm value
Negative Control (NC) of product code SAG1.CE/SAG1ULTRA.CE	< 0.050 mean OD450nm value after blanking
Calibrator (CAL) of product code SAG1.CE/SAG1ULTRA.CE treated with CONTROL	S/Co $\geq$ 2
Calibrator (CAL) of product code SAG1.CE/SAG1ULTRA.CE treated with SOLN NEUT	C/N > 2
Sample to be confirmed treated with CONTROL	S/Co > 1.1

If the results of the test match the requirements stated above, proceed to the next section.

If they do not, do not proceed any further and perform the following checks:

Problem	Check
Blank well > 0.100 OD450nm	that the Chromogen/Substrate solution has not become contaminated during the assay
Negative Control (NC) > 0.050 OD450nm after blanking	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 the negative one); 4. that no contamination of the negative control or of the wells where the control was dispensed has occurred due to spills of positive samples or of 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 (CAL) Treated with CONTROL S/Co < 2	1. that the procedure has been correctly performed; 2. that no mistake has occurred during the distribution of the Calibrator (dispensation of negative control instead) or the CONTROL (dispensation of the SOLN NEUT instead). 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination has occurred.
Calibrator (CAL) treated with SOLN NEUT C/N < 2	1. that the procedure has been correctly performed; 2. that no mistake has occurred during the distribution of the Calibrator (dispensation of negative control instead) or the SOLN NEUT (dispensation of the CONTROL instead). 3. that the washing procedure and the washer settings are as validated in the pre qualification study; 4. that no external contamination has occurred.
Sample to be confirmed treated with CONTROL S/Co < 1.1	1. the sample to be confirmed was mishandled or confused with a negative one. 2. that the SOLN NEUT was dispensed instead of the CONTROL. 3. that the washing procedure and the washer settings are as validated in the pre qualification study.

If any of the above problems have occurred, report the problem to the supervisor for further actions.

N. EXAMPLE OF RESULTS

Below an example of calculation and interpretation of results is reported:

Sample # 1

Control well C: 1.000 OD450nm
Neutralization well N: 0.100 OD450nm
Ratio C/N: 10
Result of confirmation: **true positive**

Sample # 2

Control well C: 1.000 OD450nm
Neutralization well N: 0.800 OD450nm
Ratio C/N: 1.25
Result of confirmation: **false positive**

Important note:

*If the OD450nm value for the control well (**C**) of the 1:100,000 dilution of the sample is still higher than the upper limit of detection of the microplate reader, the specimen is confirmed positive if the value for the neutralization well (**N**) is equal or less than 50% of the maximum optical density of the reader. An example of such case is reported below:*

Upper limit of detection of the reader: 2.000

Sample # 1 diluted 1:100,000

Control well C: >2.000 OD450nm
Neutralization well N: 0.800 OD450nm
50% of *the upper limit of detection of the reader*:: 1.000
Result of confirmation: ***true positive***

Sample # 2 diluted 1:100,000

Control well C: >2.000 OD450nm
Neutralization well N: 1.850 OD450nm
50% of *the upper limit of detection of the reader*::1.000
Result of confirmation: ***false positive***

O. TEST PERFORMANCES

Sensitivity:

A total of 300 samples positive for HBsAg in HBsAg One, including standards for HBsAg provided by WHO, NIBSC and PEI, were examined. In the study also 15 panels of HBsAg seroconversion were included.
All the positive samples were confirmed positive providing a value of 100% sensitivity.

Specificity:

A total of 20 false positive samples (prevalently HAMA positive), obtained from a population examined without the HAMA blocker with the kits SAG1.CE/SAG1ULTRA.CE, were tested.
All of them, again tested with a SAG1.CE/SAG1ULTRA.CE device lacking the HAMA blocker, were not confirmed for HBsAg presence and therefore defined false positives.
In addition, even if the assay is not suitable to test negative samples, a total of 50 specimens negative in HBsAg One first screening, coming from hospitalized patients with pathologies different from HBV infection, showed a mean C/N value < 2 in the confirmation assay, therefore proving the validity of the above calculation and not to generate interferences in the confirmation test.

P. LIMITATIONS OF THE TEST

All the limitations reported in the kit HBsAg One apply to the above described assay, as they are conducible to the HBsAg assay itself.
Please read with attention the Instructions for Use of the product code SAG1.CE/SAG1ULTRA.CE before carrying out the test of neutralization.

In particular the product must not be applied to those specimens showing particles or aggregates, unless the sample is cleaned before use by filtration on 0.2-0.8 u disposable filters.

The confirmation assay for HBsAg positivity is not suitable to confirm negativity on negative samples and therefore must not be used for such analysis.

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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.

Produced by
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CE
0318

HBsAg one

Version ULTRA

Fourth generation Enzyme Immunoassay (ELISA) for the determination of Hepatitis B surface Antigen or HBsAg in human serum and plasma

- for “in vitro” diagnostic use only -

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REF SAG1ULTRA.CE
96/192/480/960 Tests

HBsAg One version ULTRA

A. INTENDED USE

Fourth generation Enzyme Immunoassay (ELISA) for the one-step determination of Hepatitis B surface Antigen or HBsAg in human plasma and sera.
The kit is intended for the screening of blood units, is able to detect HBsAg mutants and finds application in the follow-up of HBV-infected patients.
For "in vitro" diagnostic use only.

B. INTRODUCTION

The World Health Organization (WHO) defines Hepatitis B Virus infection as follows:

"Hepatitis B is one of the major diseases of mankind and is a serious global public health problem. Hepatitis means inflammation of the liver, and the most common cause is infection with one of 5 viruses, called hepatitis A,B,C,D, and E. All of these viruses can cause an acute disease with symptoms lasting several weeks including yellowing of the skin and eyes (jaundice); dark urine; extreme fatigue; nausea; vomiting and abdominal pain. It can take several months to a year to feel fit again. Hepatitis B virus can cause chronic infection in which the patient never gets rid of the virus and many years later develops cirrhosis of the liver or liver cancer.

HBV is the most serious type of viral hepatitis and the only type causing chronic hepatitis for which a vaccine is available. Hepatitis B virus is transmitted by contact with blood or body fluids of an infected person in the same way as human immunodeficiency virus (HIV), the virus that causes AIDS. However, HBV is 50 to 100 times more infectious than HIV. The main ways of getting infected with HBV are: (a) perinatal (from mother to baby at the birth); (b) child- to-child transmission; (c) unsafe injections and transfusions; (d) sexual contact.

Worldwide, most infections occur from infected mother to child, from child to child contact in household settings, and from reuse of un-sterilized needles and syringes. In many developing countries, almost all children become infected with the virus. In many industrialized countries (e.g. Western Europe and North America), the pattern of transmission is different. In these countries, mother-to-infant and child-to-child transmission accounted for up to one third of chronic infections before childhood hepatitis B vaccination programmes were implemented. However, the majority of infections in these countries are acquired during young adulthood by sexual activity, and injecting drug use. In addition, hepatitis B virus is the major infectious occupational hazard of health workers, and most health care workers have received hepatitis B vaccine.

Hepatitis B virus is not spread by contaminated food or water, and cannot be spread casually in the workplace. High rates of chronic HBV infection are also found in the southern parts of Eastern and Central Europe. In the Middle East and Indian sub-continent, about 5% are chronically infected. Infection is less common in Western Europe and North America, where less than 1% are chronically infected.

Young children who become infected with HBV are the most likely to develop chronic infection. About 90% of infants infected during the first year of life and 30% to 50% of children infected between 1 to 4 years of age develop chronic infection. The risk of death from HBV-related liver cancer or cirrhosis is approximately 25% for persons who become chronically infected during childhood. Chronic hepatitis B in some patients is treated with drugs called *interferon or lamivudine*, which can help some patients. Patients with cirrhosis are sometimes given liver transplants, with varying success. It is preferable to prevent this disease with vaccine than to try and cure it.

Hepatitis B vaccine has an outstanding record of safety and effectiveness. Since 1982, over one billion doses of hepatitis B vaccine have been used worldwide. The vaccine is given as a series of three intramuscular doses. Studies have shown that the vaccine is 95% effective in preventing children and adults from developing chronic infection if they have not yet been infected. In many countries where 8% to 15% of children used to become chronically infected with HBV, the rate of chronic infection has been reduced to less than 1% in immunized groups of children. Since 1991, WHO has called for all countries to add hepatitis B vaccine into their national immunization programs."

Hepatitis B surface Antigen or HBsAg is the most important protein of the envelope of Hepatitis B Virus, responsible for acute and chronic viral hepatitis.
The surface antigen contains the determinant "a", common to all the known viral subtypes, immunologically distinguished by two distinct subgroups (ay and ad).
The ability to detect HBsAg with high sensitive immunoassays in the last years has led to an understanding of its distribution and epidemiology worldwide and to radically decrease the risk of infection in transfusion.

C. PRINCIPLE OF THE TEST

A mix of mouse monoclonal antibodies specific to the determinants "a", "d" and "y" of HBsAg is fixed to the surface of microwells. Patient's serum/plasma is added to the microwell together with a second mix of mouse monoclonal antibodies, conjugated with Horseradish Peroxidase (HRP) and directed against a different epitope of the determinant "a" and against "preS".
The specific immunocomplex, formed in the presence of HBsAg in the sample, is captured by the solid phase.
At the end of the one-step incubation, microwells are washed to remove unbound serum proteins and HRP conjugate.
The chromogen/substrate is then added and, in the presence of captured HBsAg immunocomplex, the colorless substrate is hydrolyzed by the bound HRP conjugate to a colored end-product. After blocking the enzymatic reaction, its optical density is measured by an ELISA reader.
The color intensity is proportional to the amount of HBsAg present in the sample.
The version ULTRA is particularly suitable for automated screenings and is able to detect "s" mutants.

D. COMPONENTS

The standard configuration contains reagents to perform 192 tests and is made of the following components:

1. Microplate MICROPLATE
n° 2. 12 strips of 8 breakable wells coated with anti HBsAg, affinity purified mouse monoclonal antibodies, specific to "a", "y" and "d" determinants, and sealed into a bag with desiccant.

2. Negative Control CONTROL -
1x4.0ml/vial. Ready to use control. It contains goat serum, 10 mM phosphate buffer pH 7.4+/-0.1, 0.09% Na-azide and 0.045% ProClin 300 as preservatives. The negative control is pale yellow color coded.

3. Positive Control CONTROL +
1x4.0ml/vial. Ready to use control. It contains goat serum, non infectious recombinant HBsAg, 10 mM phosphate buffer pH 7.4+/-0.1, 0.02% gentamicine sulphate and 0.045% ProClin 300 as preservatives. The positive control is color coded green.

4. Calibrator CAL ….
n° 2 vials. Lyophilized calibrator. To be dissolved with EIA grade water as reported in the label. Contains fetal bovine serum, non infectious recombinant HBsAg at 0.5 IU/ml (2nd WHO international standard for HBsAg, NIBSC code 00/588), 10 mM phosphate buffer pH 7.4+/-0.1, 0.02% gentamicine sulphate and 0.045% ProClin 300 as preservatives.
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 .

5. Wash buffer concentrate WASHBUF 20X
2x60ml/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.

6. Enzyme Conjugate Diluent CONJ DIL
2x16ml/vial. Ready to use and pink/red color coded regent. It contains 10 mM Tris buffer pH 6.8+/-0.1, 1% normal mouse serum, 5% BSA, 0.045% ProClin 300 and 0.02% gentamicine sulphate as preservatives. The solution is normally opalescent.

7. Enzyme Conjugate CONJ 20X
2x1ml/vial. 20X concentrated reagent. It contains Horseradish Peroxidase (HRP) labeled mouse monoclonal antibodies to HBsAg, determinant "a" and "preS", 10 mM Tris buffer pH 6.8+/-0.1, 5% BSA, 0.045% ProClin 300 and 0.02% gentamicine sulphate as preservatives.

8. Chromogen/Substrate SUBS TMB
2x25ml/bottle. It contains a 50 mM citrate-phosphate buffered solution at pH 3.5-3.8, 4% dimethylsulphoxide, 0.03% tetra-methyl-benzidine (TMB) and 0.02% hydrogen peroxide (H2O2).
Note: To be stored protected from light as sensitive to strong illumination.

9. Sulphuric Acid H_2SO_4 O.3 M
1x25ml/bottle. It contains 0.3 M H2SO4 solution.
Note: Attention: Irritant (H315; H319; P280; P302+P352; P332+P313; P305+P351+P338; P337+P313; P362+P363)

10. Plate sealing foils n° 4

11. Package insert

Important note:
Only upon specific request , Dia.Pro can supply reagents for 96, 480, 960 tests , as reported below**:**

Microplates	N°1	N°5	N°10
Negative Control	1x2ml/vial	1x10ml/vial	1x20ml/vial
Positive Control	1x2ml/vial	1x10ml/vial	1x20ml/vial
Calibrator	N° 1 vial	N° 5 vials	N° 10 vials
Wash buffer concentrate	1x60ml/vial	5x60ml/vial	4x150ml/vial
Enzyme conjugate	1x0.8ml/vial	1x4ml/vial	2x4ml/vial
Conjugate Diluent	1x16ml/vial	2x40ml/vial	2x80ml/vial
Chromogen/Substrate	1x25ml/vial	3x42ml/vial	2x125ml/vial
Sulphuric Acid	1x15ml/vial	2x40ml/vial	2x80ml/vial
Plate sealing foils	N° 2	N° 10	N° 20
Package insert	N° 1	N° 1	N° 1
Number of tests	96	480	960
Code SAG1ULTRA.CE	96	480	960

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), capable to provide shaking at 1300 rpm+/-150, 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. When the kit is used for the screening of blood units and blood components, it has to be used in a laboratory certified and qualified by the national authority in that field (Ministry of Health or similar entity) to carry out this type of analysis.
3. 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.
4. All the personnel involved in sample handling should be vaccinated for HBV and HAV, for which vaccines are available, safe and effective.
5. 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 (TMB) from strong light and avoid vibration of the bench surface where the test is undertaken.
6. Upon receipt, store the kit at 2..8°C into a temperature controlled refrigerator or cold room.
7. 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.
8. 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 for kit replacement.
9. Avoid cross-contamination between serum/plasma samples by using disposable tips and changing them after each sample. Do not reuse disposable tips.
10. Avoid cross-contamination between kit reagents by using disposable tips and changing them between the use of each one. Do not reuse disposable tips.
11. Do not use the kit after the expiration date stated on the external container and internal (vials) labels. A study conducted on an opened kit has not pointed out any relevant loss of activity up to 6 re-use of the device and up to 6 months.
12. 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.
13. The use of disposable plastic-ware is recommended in the preparation of the liquid components or in transferring components into automated workstations, in order to avoid cross contamination.
14. 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 before waste. 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..
15. Accidental spills from samples and operations 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.
16. The Stop Solution is an irritant. In case of spills, wash the surface with plenty of water
17. 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 WARNINGS

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 to samples; 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. When the kit is used for the screening of blood units, bar code labeling and electronic reading is strongly recommended.
4. Haemolysed (red) and lipemic ("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 well as they could give rise to false positive results. Specimens with an altered pathway of coagulation, presenting particles after blood collection and preparation of serum/plasma as those coming from hemodialized patients, could give origin to false positive 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 sample should not be frozen/thawed more than once as this may generate particles that could affect the test result.
6. If some turbidity is present or presence of microparticles is suspected after thawing, filter the sample on a disposable 0.2-0.8u filter to clean it up for testing or use the two-steps alternative method.

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 6 months.

1. Microplates:
Allow the microplate to reach room temperature (about 1 hr) before opening the container. Check that the desiccant has not turned green, indicating a defect in conservation.
In this case, call Dia.Pro's customer service.
Unused strips have to be placed back inside the aluminum pouch, with the desiccant supplied, firmly zipped and stored at +2°..8°C. After first opening, remaining 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. Positive Control:
Ready to use. Mix well on vortex before use. The positive control does not contain any infective HBV as it is composed of recombinant synthetic HBsAg.

4. 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. The solution is not stable. Store the Calibrator frozen in aliquots at –20°C.

5. Wash buffer concentrate:
The 20x concentrated solution has to be diluted with EIA grade water up to 1200 ml and mixed gently end-over-end before use. As some salt crystals may be present into the vial, take care to dissolve all the content when preparing the solution.
In the preparation avoid foaming as the presence of bubbles could give origin to a bad washing efficiency.

Note: *Once diluted, the wash solution is stable for 1 week at +2..8° C.*

6. Enzyme conjugate:
The working solution is prepared by diluting the 20X concentrated reagent into the Conjugate
Mix well on vortex before use.
Avoid any contamination of the liquid with oxidizing chemicals, dust or microbes. If this component has to be transferred, use only plastic sterile disposable containers.
Important note*: The working solution is not stable. Prepare only the volume necessary for the work of the day. As an example when the kit is used in combination with other instruments or manually, dilute 0.1 ml 20X Conjugate with 1.9 ml Conjugate Diluent into a disposable plastic vial and mix carefully before use.*

7. Chromogen/Substrate:
Ready to use. Mix well by end-over-end mixing.
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.

8. Sulphuric Acid:
Ready to use. Mix well by end-over-end mixing.
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 (70% ethanol, 10% solution of bleach, hospital grade disinfectants) of those parts that could accidentally come in contact with the sample or the components of the kit. 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 $\pm$1°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. In case of **shaking** during incubations, the instrument has to ensure 350 rpm $\pm$150. Amplitude of shaking is very important as a wrong one could give origin to splashes and therefore to some false positive result.
4. 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.

5. **Incubation times** have a tolerance of ±5%.
6. The **microplate reader** has to be equipped with a reading filter of 450nm and with a second filter of 620-630nm, mandatory for blanking purposes. Its standard performances should be (a) bandwidth ≤ 10 nm; (b) absorbance range from 0 to ≥ 2.0; (c) linearity to ≥ 2.0; (d) repeatability ≥ 1%. 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 that the correct optical density is measured. It should be regularly maintained according to the manufacturer 's instructions.
7. When using **ELISA automated workstations**, all critical steps (dispensation, incubation, washing, reading, shaking, data handling, etc.) have to be carefully set, calibrated, controlled and regularly serviced in order to match the values reported in the sections "Internal Quality Control". The assay protocol has to be installed in the operating system of the unit and validated by checking full matching the declared performances of the kit. In addition, the liquid handling part of the station (dispensation and washing) has to be validated and correctly set paying particular attention to avoid carry over by the needles used for dispensing samples and for washing. The carry over effect must be studied and controlled to minimize the possibility of contamination of adjacent wells due to strongly reactive samples, leading to false positive results. The use of ELISA automated work stations is recommended for blood screening and when the number of samples to be tested exceed 20-30 units per run.
8. When using automatic devices, in case the vial holder of the instrument does not fit with the vials supplied in the kit, transfer the solution into appropriate containers and label them with the same label peeled out from the original vial. This operation is important in order to avoid mismatching contents of vials, when transferring them. When the test is over, return the secondary labeled containers to 2..8°C, firmly capped.
9. **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 full compliance with the essential requirements of the assay. Support is also provided for the installation of new instruments to be used in combination with the kit.

L. PRE ASSAY CONTROLS AND OPERATIONS

1. Check the expiration date of the kit printed on the external label of the kit box. Do not use if expired.
2. Check that the liquid components are not contaminated by naked-eye visible particles or aggregates. Check that the Chromogen/Substrate is colorless or pale blue. Check that no breakage occurred in transportation and no spillage of liquid is present inside the box. Check that the aluminum pouch, containing the microplate, is not punctured or damaged.
3. Dilute all the content of the 20x concentrated Wash Solution as described above.
4. Dilute the 20X concentrated Enzyme Conjugate with its Diluent as reported.
5. Dissolve the Calibrator as described above.
6. Allow all the other components to reach room temperature (about 1 hr) and then mix as described.
7. 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.
8. Check that the ELISA reader has been turned on at least 20 minutes before reading.
9. If using an automated workstation, turn it on, check settings and be sure to use the right assay protocol.
10. Check that the micropipettes are set to the required volume.
11. Check that all the other equipment is available and ready to use.
12. In case of problems, do not proceed further with the test and advise the supervisor.

M. ASSAY PROCEDURE

The assay has to be carried out according to what reported below, taking care to maintain the same incubation time for all the samples in testing.

Automated assay:

In case the test is carried out automatically with an ELISA system, we suggest to make the instrument dispense first 150 ul controls & calibrator, then all the samples and finally 100 ul diluted Enzyme Conjugate.
For the pre-washing step (point 1 of the assay procedure) and all the next operations follow the operative instructions reported below for the Manual Assay.
It is strongly recommended to check that the time lap between the dispensation of the first and the last sample will be calculated by the instrument and taken into consideration by delaying the first washing operation accordingly.

Manual Assay:

1. Place the required number of strips in the plastic holder and wash them once to hydrate wells. Carefully identify the wells for controls, calibrator and samples.

Important note: *Pre washing (1 cycle: dispensation of 350ul/well of washing solution+ aspiration) is fundamental to obtain reliable and specific results both in the manual and in the automatic procedures. Do not omit it !*

2. Leave the A1 well empty for blanking purposes.
3. Pipette 150µl of the Negative Control in triplicate, 150ul of the Calibrator in duplicate and then 150ul of the Positive Control in single followed by 150ul of each of the samples.
4. 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).
5. Dispense 100ul diluted Enzymatic 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 when the conjugate is dispensed. Contamination might occur.*

6. Following addition of the conjugate, check that the color of the samples have changed from yellowish to pink/red and then incubate the microplate for **120 min at +37°C**.

Important notes:

a. 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.
b. If the procedure is carried out on shaking, be sure to deliver the rpm reported for in Section I.3 as otherwise intra-well contamination could occur.

7. When the first incubation is over, wash the microwells as previously described (section I.4)
8. Pipette 200 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.*

9. Incubate the microplate protected from light at **18-24°C for 30 min.** Wells dispensed with the positive control, the calibrator and positive samples will turn from clear to blue.
10. Pipette 100 µl Sulphuric Acid into all the wells to stop the enzymatic reaction, using the same pipetting sequence as in step 8. Addition of the acid solution will turn the positive control, the calibrator and positive samples from blue to yellow/brown.
11. Measure the color intensity of the solution in each well, as described in section I.6 using a 450nm filter (reading) and a 620-630nm filter (background subtraction, mandatory), blanking the instrument on A1.

Important general notes:

1. *Ensure that no fingerprints or dust are present on the external bottom of the microwell before reading. They could generate false positive results on reading.*
2. *Reading should ideally be performed immediately after the addition of the acid solution but definitely no longer than 20 minutes afterwards. Some self-oxidation of the chromogen can occur leading to a higher background.*
3. *When samples to be tested are not surely clean or have been stored frozen, the assay procedure reported below is recommended as long as it is far less sensitive to interferences due to hemolysis, hyperlipaemia, bacterial contamination and fibrin microparticles. The assay is carried out in two-steps at +37°C on shaking at* 350 rpm ±150 *as follows:*
   - *dispense 100 ul of controls, calibrator and samples*
   - *incubate 60 min at +37°C on shaking*
   - *wash according to instructions (section I.4)*
   - *dispense 100 ul diluted enzyme tracer*
   - *incubate 30 min at +37°C on shaking*
   - *wash*
   - *dispense 100 ul TMB&H2O2 mix*
   - *incubate 30 min at r.t. on shaking*
   - *stop and read*

   In this procedure the pre-wash can be omitted.
   This method shows performances similar to the standard one and therefore can be used in alternative.
4. *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

Operations	Procedure
Pre-Washing step	n° 1 cycle
Controls&Calibrator&samples Diluted Enzyme Conjugate	150 ul 100 ul
1st incubation	**120 min**
Temperature	+37°C
Washing steps	n° 5 cycles with 20'' of soaking OR n° 6 cycles without soaking
Chromogen/Substrate	200ul
2nd incubation	**30 min**
Temperature	room
Sulphuric Acid	100 ul
Reading OD	450nm / 620-630nm

An example of dispensation scheme is reported in the following section:

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
CAL = Calibrator PC = Positive Control S = Sample

O. INTERNAL QUALITY CONTROL

A check is performed on the controls/calibrator any time the kit is used in order to verify whether the expected OD450nm or S/Co values have been matched in the analysis.

Ensure that the following results are met:

Parameter	Requirements
Blank well	< 0.100 OD450nm value
Negative Control (NC)	< 0.050 mean OD450nm value after blanking
Calibrator 0.5 IU/ml	S/Co ≥ 2
Positive Control	> 1.000 OD450nm value

If the results of the test match the requirements stated above, proceed to the next section.
If they do not, do not proceed any further and perform the following checks:

Problem	Check
Blank well > 0.100 OD450nm	1. that the Chromogen/Substrate solution has not become contaminated during the assay
Negative Control (NC) > 0.050 OD450nm after blanking	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 the negative one); 4. that no contamination of the negative control or of the wells where the control was dispensed has occurred due to spills of positive samples or of 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 of calibrator) 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 < 1.000 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. In this case, the negative control will have an OD450nm value > 0.050). 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 11.

P. CALCULATION OF THE CUT-OFF
The test results are calculated by means of a cut-off value determined on the mean OD450nm/620-630nm value of the negative control (NC) with the following formula:

NC + 0.050 = Cut-Off (Co)

The value found for the test is used for the interpretation of results as described in the next paragraph.

***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
Test results are interpreted as a ratio of the sample OD450nm/620-630nm (S) and the Cut-Off value (Co), mathematically S/Co, according to the following table:

S/Co	Interpretation
< 0.9	Negative
0.9 – 1.1	Equivocal
> 1.1	Positive

A negative result indicates that the patient is not infected by HBV and that the blood unit may be transfused.
Any patient showing an equivocal result should be retested on a second sample taken 1-2 weeks after the initial sample; the blood unit should not be transfused.
A positive result is indicative of HBV infection and therefore the patient should be treated accordingly or the blood unit should be discarded.

Important notes:
1. *Interpretation of results should be done under the supervision of the laboratory supervisor to reduce the risk of judgment errors and misinterpretations.*
2. *Any positive result must be confirmed first by repeating the test on the sample, after having filtered it on 0.2-0.8 u filter to remove any microparticles interference. Then, if still positive, the sample has to be submitted to a confirmation test before a diagnosis of viral hepatitis is released.*
3. *When test results are transmitted from the laboratory to another department, attention must be paid to avoid erroneous data transfer.*
4. *Diagnosis of viral hepatitis infection has to be taken and released to the patient by a suitably qualified medical doctor.*

An example of calculation is reported below (data obtained proceeding as the the reading step described in the section M, point 11):

The following data must not be used instead or real figures obtained by the user.

Negative Control: 0.012 – 0.008 – 0.010 OD450nm
Mean Value: 0.010 OD450nm
Lower than 0.050 – Accepted
Positive Control: 2.489 OD450nm
Higher than 1.000 – Accepted
Cut-Off = 0.010+0.050 = 0.060
Calibrator: 0.350 - 0.370 OD450nm
Mean value: 0.360 OD450nm S/Co = 6.0
S/Co higher than 2.0 – Accepted
Sample 1: 0.028 OD450nm
Sample 2: 1.690 OD450nm
Sample 1 S/Co < 0.9 = negative
Sample 2 S/Co > 1.1 = positive

R. PERFORMANCE CHARACTERISTICS
Evaluation of Performances has been conducted in accordance to what reported in the Common Technical Specifications or CTS (art. 5, Chapter 3 of IVD Directive 98/79/EC). Version ULTRA proved to be at least equivalent to the original design in a study conducted for the validation of the new version.

1. Analytical Sensitivity
The limit of detection of the assay has been calculated on the 2nd WHO international standard, NIBSC code 00/588.
In the following table, results are given for three lots (P1, P2 and P3) of the version ULTRA in comparison with the reference device (Ref.):

WHO IU/ml	Lot # P1 S/Co	Lot # P2 S/Co	Lot # P3 S/Co	Ref. S/Co
0.4	**4.6**	**4.8**	**4.6**	**4.6**
0.2	**2.3**	**2.4**	**2.4**	**2.4**
0.1	**1.4**	**1.4**	**1.5**	**1.2**
0.05	0.8	0.8	***1.0***	0.7
0.025	0.6	0.6	0.6	0.4
FCS (NC)	0.3	0.2	0.3	0.1

The assay shows an Analytical Sensitivity better than 0.1 WHO IU/ml of HBsAg.

In addition two panels of sensitivity supplied by EFS, France, and by SFTS, France, were tested and gave in the best conditions the following results:

Panel EFS Ag HBs HB1-HB6 lot n° 04

Sample ID	Characteristics	ng/ml	S/Co
HB1	diluent	/	0,2
HB2	adw2+ayw3	0.05	0,6
HB3	adw2+ayw3	0.1	**1,0**
HB4	adw2+ayw3	0.2	**1,8**
HB5	adw2+ayw3	0.3	**2,4**
HB6	adw2+ayw3	0.5	**4,2**

Sensitivity panel SFTS, France, Ag HBs 2005

Sample ID	Characteristics	ng/ml	S/Co
171	Adw2 + ayw3	2.21 ± 0.15	**15,4**
172	Adw2 + ayw3	1.18 ± 0.10	**8,7**
173	Adw2 + ayw3	1.02 ± 0.05	**6,1**
174	Adw2 + ayw3	0.64 ± 0.04	**4,0**
175	Adw2 + ayw3	0.49 ± 0.03	**3,4**
176	Adw2 + ayw3	0.39 ± 0.02	**2,6**
177	Adw2 + ayw3	0.25 ± 0.02	**2,0**
178	Adw2 + ayw3	0.11 ± 0.02	**1,3**
179	Adw2 + ayw3	0.06 ± 0.01	***0,9***
180	Adw2 + ayw3	0.03 ± 0.01	0,8
181	Adw2	0.5 – 1.0	**4,7**
182	Adw4	0.5 – 1.0	**3,6**
183	Adr	0.5 – 1.0	**4,5**
184	Ayw1	0.5 – 1.0	**5,1**
185	Ayw2	0.5 – 1.0	**6,4**
186	Ayw3	0.5 – 1.0	**7,3**
187	Ayw3	0.5 – 1.0	**5,8**
188	Ayw4	0.5 – 1.0	**6,9**
189	Ayr	0.5 – 1.0	**6,1**
190	diluent	/	0,6

The panel # 808, supplied by Boston Biomedical Inc., USA, was also tested to define the limit of sensitivity.
Results in the best conditions are as follows :

BBI panel PHA 808

Sample ID	Characteristics	ng/ml	S/Co
01	ad	2,49	**10,2**
02	ad	1,17	**4,8**
03	ad	1,02	**4,3**
04	ad	0,96	**3,8**
05	ad	0,69	**2,9**
06	ad	0,50	**2,2**
07	ad	0,41	**1,5**
08	ad	0,37	**1,3**
09	ad	0,30	**1,2**
10	ad	0,23	***1,0***
11	ay	2,51	**11,2**
12	ay	1,26	**5,9**
13	ay	0,97	**4,1**
14	ay	0,77	**3,7**
15	ay	0,63	**2,0**
16	ay	0,48	**2,4**
17	ay	0,42	**2,0**
18	ay	0,33	**1,8**
19	ay	0,23	**1,6**
20	ay	0,13	**1,1**
21	negative	/	0,6

2. Diagnostic Sensitivity:
The diagnostic sensitivity was tested according to what required by Common Technical Specifications (CTS) of the directive 98/79/EC on IVD for HBsAg testing.
Positive samples, including HBsAg subtypes and a panel of "s" mutants from most frequent mutations, were collected from different HBV pathologies (acute, a-symptomatic and chronic hepatitis B) or produced synthetically, and were detected positive in the assay.
All the HBsAg known subtypes, "ay" and "ad", and isoforms "w" and "r", supplied by CNTS, France, were tested in the assay and determined positive by the kit as expected.
An overall value of 100% has been found in a study conducted on a total number of more than 400 samples positive with the original reference IVD code SAG1.CE, CE marked.
A total of 30 sero-conversions were studied, most of them produced by Boston Biomedica Inc., USA.
Results obtained by examining eight panels supplied by Boston Biomedica Inc., USA, are reported below for the version ULTRA in comparison with the reference device code SAG1.CE.

Panel ID	1st sample positive	HBsAg subtype	HBsAg ng/ml	Version ULTRA S/Co	Ref. device S/Co
PHM 906	02	ad	0.5	**3.7**	**1.4**
PHM 907 (M)	06	ay	1.0	**4.4**	**2.9**
PHM 909	04	ad	0.3	**1.2**	0.8
PHM 914	04	ad	0.5	**1.1**	**1.1**
PHM 918	02	ad	0.1	**1.8**	0.5
PHM 923	03	ay	< 0.2	**2.2**	**1.2**
PHM 925	03	Ind.	n.d.	**1.4**	*0.9*
PHM 934	01	ad	n.d.	**1.0**	0.8

3. Diagnostic Specificity:
It is defined as the probability of the assay of scoring negative in the absence of specific analyte. In addition to the first study, where more than 5000 negative samples from blood donors (two blood centers), classified negative with a CE marked device in use at the laboratory of collection were examined, the diagnostic specificity was recently assessed by testing a total of 2288 negative blood donors on seven different lots. A value of specificity of 100% was found.
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 samples freezing 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, HAV) and non viral pathologies of the liver that may interfere with the test were examined. No cross reaction were observed.

4. Precision:
It has been calculated for the version ULTRA on two samples examined in 16 replicates in 3 different runs for three lots.
Results are reported in the following tables:

Average values Total n = 144	Negative Sample	Calibrator 0.5 IU/ml
OD450nm	0.026	0.332
Std.Deviation	0.004	0.027
CV %	16%	8%

The variability shown in the tables did not result in sample misclassification.

S. LIMITATIONS

Repeatable false positive results were assessed on freshly collected specimens in less than 0.1% of the normal population, mostly due to high titers Heterophilic Anti Mouse Antibodies (HAMA).

Interferences in fresh samples were also observed when they were not particles-free or were badly collected (see chapter G).

Old or frozen samples, presenting fibrin clots, crioglobulins, lipid-containing micelles or microparticles after storage or thawing, can generate false positive results.

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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:
Dia.Pro Diagnostic Bioprobes S.r.l.
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CE
0318

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 (E)
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 (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 2-8ºC. 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 2-8ºC**
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).

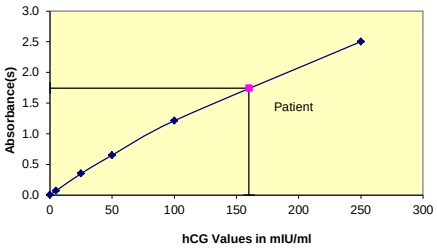
Note: Computer data reduction software designed for ELISA assays may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained.

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).
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4. Goldstein DP, and Kosasa TS, "The Subunit Radioimmunoassay for HCG Clinical Application", *Gynecology, 6*, 145-84 (1975).
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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).
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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 (E)
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).

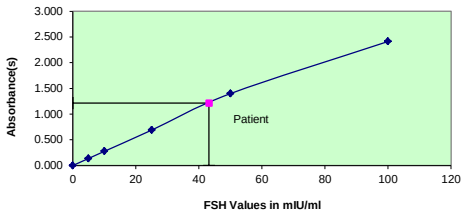
Note: Computer data reduction software designed for ELISA assays may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained.

*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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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

Luteinizing Hormone (LH) Test System
Product Code: 625-300

1.0 INTRODUCTION

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

2.0 SUMMARY AND EXPLANATION OF THE TEST

Luteinizing hormone (LH) is a glycoprotein consisting of two subunits with a molecular mass of 30,000 daltons. The α-subunit is similar to other pituitary hormones follicle stimulating hormone (FSH), thyroid stimulating hormone (TSH) and chorionic gonadotropin (CG)] while the β-subunit is unique. The β-subunit confers the biological activity to the molecule. The α-subunit consists of 89 amino acid residues while the β-subunit contains 129 amino acids. The carbohydrate content is between 15% and 30%.

The clinical usefulness of the measurement of luteinizing hormone (LH) in ascertaining the homeostasis of fertility regulation via the hypothalamic - pituitary - gonadal axis has been well established.[1,2] In addition, the advent of *in vitro* fertilization (IVF) technology to overcome infertility-associated problems has provided the impetus for rapid improvement in LH assay methodology from the technically demanding bioassay[3] to the procedurally simple and rapid immunoenzymometric assays.

In this method, LH 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 LH) are added and the reactants mixed. Reaction between the various LH antibodies and native LH forms a sandwich complex that binds with the streptavidin coated to the well.

After the completion of the required incubation period, the enzyme-luteinizing hormone antibody bound conjugate is separated from the unbound enzyme-luteinizing 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 luteinizing 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 luteinizing 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-LH 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:

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

$^{Btn}Ab_{(m)}$ = Biotinylated Monoclonal Antibody (Excess Quantity)
Ag_{LH} = Native Antigen (Variable Quantity)
$^{Enz}Ab_{(p)}$ = Enzyme labeled Antibody (Excess Quantity)
$^{Enz}Ab_{(p)}\text{-}Ag_{LH}\text{-}{}^{Btn}Ab_{(m)}$ = 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:
$^{Enz}Ab_{(p)}\text{-}Ag_{LH}\text{-}{}^{Btn}Ab_{(m)} + Streptavidin_{C.W.} \Rightarrow$ Immobilized complex
$Streptavidin_{C.W.}$ = Streptavidin immobolized on well
Immobilized complex = Antibodies-Antigen sandwich bound

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. LH Calibrators – 1ml/vial – Icons A-F
Six (6) vials of references for LH Antigen at levels of 0(**A**), 5(**B**), 25(**C**), 50(**D**), 100(**E**) and 200(**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 IS 80/552.

B. LH 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 capable of delivering 0.050ml (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 gel barrier. 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, 0.100 ml (100 µl) 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 LH-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 luteinizing hormone (LH) 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 LH 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 LH 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 (1.005) intersects the dose response curve at 42.7 mIU/ml LH 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.

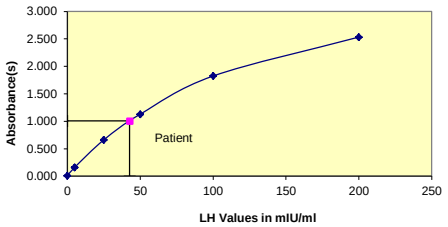
*The data presented in Example 1 and Figure 1 is for illustration only and **should not** be used in lieu of a dose response curve prepared with each assay.

EXAMPLE 1

Sample I.D.	Well Number	Abs (A)	Mean Abs (B)	Value (mIU/ml)
Cal A	A1	0.009	0.009	0
	B1	0.009		
Cal B	C1	0.161	0.162	5
	D1	0.163		
Cal C	E1	0.677	0.662	25
	F1	0.647		
Cal D	G1	1.155	1.130	50
	H1	1.106		
Cal E	A2	1.852	1.825	100
	B2	1.797		
Cal F	C2	2.556	2.534	200
	D2	2.512		
Ctrl 1	E2	0.077	0.072	**1.9**
	F2	0.067		
Ctrl 2	G2	0.582	0.575	**20.5**
	H2	0.568		
Patient	A3	0.998	1.005	**42.7**
	B3	1.112		

*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 the 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'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 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. LH 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.
8. Luteinizing hormone is 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 LH AccuBind® ELISA Test System. The expected values are presented in Table 1.

TABLE I
Expected Values for the LH ELISA Test System (in mIU/ml)

Women			
Follicular phase	0.5	--	10.5
Midcycle	18.4	--	61.2
Luteal phase	0.5	--	10.5
Postmenopausal	8.2	--	40.8
Men			
0.7	**--**	**7.4**	

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 LH 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 mIU/ml)

Sample	N	X	σ	C.V.
Level 1	20	1.4	0.10	6.8%
Level 2	20	21.6	0.85	3.9%
Level 3	20	58.3	2.10	3.6%

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

Sample	N	X	σ	C.V.
Level 1	20	1.6	0.12	7.8%
Level 2	20	21.5	2.32	10.8%
Level 3	20	55.4	5.34	9.6%

*As measured in ten experiments in duplicate.

14.2 Sensitivity

The LH AccuBind® ELISA Test System has a sensitivity of 0.003mIU/well. This is equivalent to a sample containing 0.054 mIU/ml LH concentration. The analytical 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 LH AccuBind® ELISA Test System 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 LH ELISA method 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
This Method	14.8	y = 0.081 + 0.93(x)	0.989
Reference	15.1		

Only slight amounts of bias between the LH 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 LH 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 Luteinizing Hormone needed to produce the same absorbance.

Substance	Cross Reactivity	Concentration
Lutropin (LH)	1.0000	---
β-LH subunit	1.0800	---
Follitropin (FSH)	< 0.0001	1000ng/ml
Chorionic gonadotropin (CG)	< 0.0001	1000ng/ml
Thyrotropin (TSH)	< 0.0001	1000ng/ml

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Revision: 5 Date: 2019-Jul-16 DCO: 1353
Product Code: *625-300*
MP625

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
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

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 2-8ºC. 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

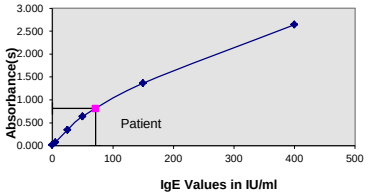
paper (do not average the duplicates of the serum references before plotting).
3. Draw the best-fit curve through the plotted points.
4. To determine the concentration of IgE for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting 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

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MP2525 Product Code: *2525-300*

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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)

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

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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IVD 2°-8° CE 0050

EC REP **CEpartner4U,** Esdoornlaan 13 3951 DBMaarn, The Netherlands **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
- 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

THYROGLOBULIN (TG) CONTROL - TRI LEVEL **PRODUCT CODE: TG-300**
LOT# TGAC1H3 **EXP: 2026-08-01**

INTENDED USE
The Thyroglobulin 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 thyroglobulin levels. This product is human-serum based, liquid control, stabilized with preservatives and can be used with all RIA, EIA, ELISA, CLIA, and FIA 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 thyroglobulin controls are intended to be used in the exact manner as patient samples. The control is packaged as 6 vials of 3.0 ml (2 of each level). 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) Aliquot the materials in 0.5 ml aliquots in cryo vials and store at ≤ -20ºC.

STORAGE, STABILITY AND DISPOSAL
The control is provided liquid and ready to use. This product will be stable until the expiration date when stored unopened at **< - 20.** Once the control is opened, all analytes will be 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.

Long term room temperature storage is not supported. Unused controls should be tightly capped and frozen within two (2) hours. Once thawed, do not refreeze the control; discard remaining material. 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
Unopened	Three (3) years	≤ -20ºC
Opened	Thirty (30) days	2-8°C

ASSIGNMENT OF VALUES & EXPECTED RANGE OF VALUES

EXPECTED RANGE OF VALUES FOR Thyroglobulin Controls - Tri-level Set MASTER LOT TGAC1H3				
Analyte	A Range	B Range	C Range	Method
Tg in ng/ml	2.44 ± 0.80	11.60 ± 3.83	57.59 ± 19.0	MB ACCUBIND ELISA
	1.78 ± 0.59	10.03 ± 3.31	52.75 ± 17.41	MB ACCULITE CLIA

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 QC 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 manufacturer's stated 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 negative and 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: 0 **Date: 2023-08-01** **Product Code: TG-300**

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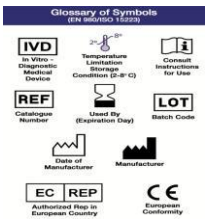

DOCUMENT HISTORY
PREPARED BY: _____ DEPT: QC VERIFIED BY: _____ DEPT:QA
APPROVED BY: _____ DEPT: Admin EFFECTIVE DATE: 2023-08-01
REVISION: 0 DCO: N/A

QSure™ Quality Control Sera

Ensure your assay results. Simple to use. Cost effective.

Monobind's QSure® Tumor Marker Control is human-serum based and designed to assist clinical laboratories with immunoassay precision. It contains cancer antigens with established value ranges for Monobind's AccuBind® ELISA and AccuLite® CLIA tests and can be used as commercial control for other manufacturer's methods.

- Tri-Level (A-C: Low, Middle and High)
- 3 year shelf life at <8°C
    - 7 day open-vial stability at 2-8°C
    - 90 days open-vial stability at <-10°C
- Liquid convenience, use like patient samples
- Extend use and reduce contamination
    - Aliquot into 0.5ml vials & freeze
    - Thaw per use (do not refreeze, discard remaining material)
- Product contains two vials of each A-C with 2ml fill volume (6 x 2ml)

Item# TMC-300

Analytes

CA 125
CA 15-3
CA 19-9

Contact us today to learn more
www.monobind.com :: sales@monobind.com

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Monobind, Inc.

SAFETY DATA SHEET

SECTION 1. IDENTIFICATION

1.1. Product Identifier(s)

Name:	Tumor Marker Control
Description:	QSure® Quality Control Sera
Code:	TMC-300
Characteristics:	Quality Control Material

1.2. Relevant identified uses of the substance or mixture and uses advised against

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.
For in vitro diagnostic use only. Not for internal or external use in humans or animals.

1.3. Details of the supplier of the safety data sheet

Manufacturer/Importer:	Manufacturer
Name or commercial name:	Monobind Inc.
Registered office:	100 North Pointe Drive, Lake Forest, California 92630, USA
Telephone number:	+1.949.951.2665
Fax number:	+1.949.951.3539
Email:	info@monobind.com
FDA Established	
Registration number:	2020726

1.4. Emergency telephone number

+1.949.951.2665 (Hours: 8 am-5 pm PST, Monday-Friday)

SECTION 2. HAZARD(S) IDENTIFICATION

2.1. Classification of the substance or mixture

None

2.2. Label elements

None

2.3. Other hazards

None

SECTION 3. COMPOSITION/INFORMATION ON INGREDIENTS

3.1. Substances and/or Mixtures

All concentrations of potentially hazardous substances or mixtures are below the specific concentration limits and M-factors for hazardous identification. As preparations, the product components are not classified as hazardous.

3.1.1. Tumor Marker Control A (Low Range)
N/A

3.1.2. Tumor Marker Control B (Middle Range)
N/A

3.1.3. Tumor Marker Control C (High Range)
N/A

SECTION 4. FIRST-AID MEASURES

4.1. Description of first aid measures

General instructions:	Immediately rinse with soap and plenty of water. Use personal protective working aids.
If inhaled:	Transport the affected person into the open air. If there are respiratory complaints, oxygen must be administered. If irritation persists, seek medical advice.
In case of skin contact:	Wash contacted area with soap and water. Remove contaminated clothing. If irritation occurs, seek medical advice.
In case of contact with eyes:	Rinse with a stream of water for at least 15 minutes. Thorough rinsing must be ensured by opening the eyelids. If irritation occurs, seek medical advice.
If ingested:	Do NOT induce vomiting. If conscious, rinse the mouth and administer a large amount of water to dilute the substance. In the case of unconsciousness, never administer anything orally. If irritation occurs, seek medical advice.

4.2. Most important symptoms and effects, both acute and delayed

No data available

4.3. Indication of any immediate medical attention and special treatment needed

No data available

SECTION 5. FIRE-FIGHTING MEASURES

5.1. Extinguishing media

Carbon dioxide, dry powder, foam, water

5.2. Special hazards arising from the substance or mixture

None

5.3. Advice for firefighters

Wear appropriate personal protective equipment and clothing. Wear self-contained breathing apparatus, if necessary.

SECTION 6. ACCIDENTAL RELEASE MEASURES

6.1. Personal precautions, protective equipment and emergency procedures

Avoid contact with skin and eyes. Wear suitable personal protective clothing.

6.2. Environmental precautions

Avoid penetration into sewerage systems, surface and ground water. Avoid soil pollution.

6.3. Methods and material for containment and cleaning up

Cover with suitable absorbing material. After removing the substance, rinse the spot of spilling thoroughly with water and soap. Dispose of waste according to all federal, state, and local regulations.

6.4. Reference to other sections

See Section 8 for personal protective equipment. See Section 13 for appropriate disposal methods.

SECTION 7. HANDLING AND STORAGE

7.1. Precautions for safe handling

Avoid spills. Avoid contact with skin, eyes and clothing. Use suitable protective means to work with the substance. Use in a well-ventilated area. Follow good manufacturing practices when using product. Do not drink, smoke, or eat in work areas.

7.2. Conditions for safe storage, including any incompatibilities

7.2.1. Kit and unopened components:
Store below -20 °C until expiration date.

7.2.2. Opened components:
Stable for seven (7) days at 2-8 °C

7.3. Specific end uses

Product procedure should be performed by a skilled individual or trained professional for in vitro diagnostic use only.

SECTION 8. EXPOSURE CONTROL/PERSONAL PROTECTION

8.1. Control parameters

No substances with occupational exposure limits.

8.2. Exposure controls

8.2.1.	Eye/face protection:	Safety glasses or goggles with side shields recommended
8.2.2.	Skin protection:	Compatible protective gloves recommended. Wash hands after properly removing and disposing of gloves.
	Other skin protection:	Laboratory coats are recommended.
8.2.3.	Respiratory protection:	No respiratory protection is required. Use product in rooms enabling good ventilation. If local exhaustion is necessary, general (forced) exhaustion is recommended.
8.2.4.	Thermal hazards:	None

SECTION 9. PHYSICAL AND CHEMICAL PROPERTIES

9.1. Information on basic physical and chemical properties

9.1.1.	Appearance:	
	Physical state (at 20 °C):	Liquid
	Colour:	Straw
9.1.2.	Odour:	Odourless
9.1.3.	Odour threshold:	Not applicable
9.1.4.	pH value:	6.8-7.4
9.1.5.	Melting point/freezing point:	Not determined
9.1.6.	Initial boiling point/ boiling range:	Not determined
9.1.7.	Flash point:	Not applicable
9.1.8.	Evaporation rate:	Not determined
9.1.9.	Flammability (solid, gas):	Not flammable
9.1.10.	Upper/lower flammability or explosive limits:	Not applicable
9.1.11.	Vapour pressure:	Not determined
9.1.12.	Vapour density:	Not determined
9.1.13.	Relative density:	Not determined
9.1.14.	Solubility:	Water soluble
9.1.15.	Partition coefficient: n-octanol/water:	Not determined
9.1.16.	Auto-ignition temperature:	Not applicable
9.1.17.	Decomposition temperature:	Not determined
9.1.18.	Viscosity:	Not determined
9.1.19.	Explosive properties:	None
9.1.20.	Oxidising properties:	Not determined

9.2. Other information

None

SECTION 10. STABILITY AND REACTIVITY

10.1. Reactivity

No known reactivity hazards associated with product

10.2. Chemical stability

Stable under recommended storage conditions

10.3. Possibility of hazardous reactions

No hazardous polymerization

10.4. Conditions to avoid

Monobind Inc.
ISO 13485 Certified Company

Excessive heat and light

10.5.Incompatible materials

Acids

10.6.Hazardous decomposition products

Not determined

SECTION 11. TOXICOLOGICAL INFORMATION:

11.1.Information on toxicological effects

11.1.1. Acute toxicity: Not determined
11.1.2. Skin corrosion/irritation: Not determined
11.1.3. Serious eye damage/irritation: Not determined
11.1.4. Respiratory or skin sensitisation: Not determined
11.1.5. Germ cell mutagenicity: Not determined
11.1.6. Carcinogenicity: No component of this product present at levels ≥ 0.1% is identified as probable, possible or confirmed human carcinogen by NTP (National Toxicology Program), IARC (International Agency for Research on Cancer), or OSHA (Occupational Safety & Health Administration)
11.1.7. Reproductive toxicity: Not determined
11.1.8. STOT-single exposure: Not determined
11.1.9. STOT-repeated exposure: Not determined
11.1.10. Aspiration hazard: Not determined
11.1.11. Information on likely routes of exposure:
If ingested: No known health effects
If inhaled: No known health effects
If contact with skin: No known health effects
If contact with eyes: No known health effects
11.1.12. Symptoms related to the physical, chemical, and toxicological characteristics: None after short or long-term exposure

SECTION 12. ECOLOGICAL INFORMATION

12.1.Toxicity

Not determined.

12.2.Persistence and degradability

Not determined

12.3.Bioaccumulative potential

Not determined

12.4.Mobility in soil

Not determined

12.5.Results of PBT and vPvB assessment

Not determined

12.6.Other adverse affects

Not determined

SECTION 13. DISPOSAL CONSIDERATIONS

13.1.Waste treatment methods

All waste disposals must be carried out in accordance with federal, state, and local legislation and administrative regulations. A licensed professional waste disposal service should be utilized to dispose of material and packaging.

SECTION 14. TRANSPORT INFORMATION

14.1.UN number

Not available

14.2.UN proper shipping name

Not available

14.3.Transport hazard class(es)

Not available

14.4.Packing group

Not available

14.5.Environmental hazards

Overland transport (ADR/RID): None
Water transport (ADN/IMDG): None
Air transport (ICAO/IATA): None

14.6.Special precautions for user

None

14.7.Transport in bulk according to Annex II of MARPOL73/78 and the IBC Code

Not applicable

SECTION 15. REGULATORY INFORMATION

15.1.Safety, health and environmental regulations/legislation specific for the substance or mixture

SARA Reporting Requirements: None
TSCA All components in product preparations are lifted on the US Toxic Substances Control Act inventory of chemicals or are exempt from listing.

This safety data sheet has been prepared to comply with the requirements of Annex II, European Community Regulation No. 1907/2006 REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals) and OSHA (Occupational Safety & Health Administration) 1910.1200, Appendix D.

15.2. Chemical safety assessment

None

SECTION 16. OTHER INFORMATION

Revision 1 (2015-MAY-05): Initial creation

The material safety data sheet contains data necessary to ensure safety and health and environmental protection in working with chemical substances. This product is a chemical substance and can be solely used by persons with chemical education at their own risk. Monobind kits are designed for biomedical research. The manufacturer has no responsibility for damage caused by unsuitable use and by disrespecting the enclosed working instructions. The above-stated information cannot be considered as complete and must be understood to be only a methodical instruction.

DOCUMENT HISTORY

PREPARED BY: _____ DEPT: Records Administration VERIFIED BY: A Shatola DEPT: QA

APPROVED BY: _____ DEPT: Administration EFFECTIVE DATE: 2015-MAY-05

REVISION: 0 DCO: N/A

Monobind Inc.
The World Resource for Diagnostic Products

100 North Pointe Drive
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Phone: 949-951-2665 Ex208 Fax: 949-951-3539

CERTIFICATE OF ANALYSIS

QUALITY CONTROL RELEASE

Product: QSure® Tumor Marker Control
Item #: TMC-300
Lot #: TMCAC1D4
Expiration Date: 2027-04-03

Bleed testing: Each unit of plasma used in this pool was tested per below by FDA approved reagent tests.

Test	Specification	Results
HIV-I/II	Non-Reactive @ Donor Level	Non-Reactive @ Donor Level
HIV-Ag	Non-Reactive @ Donor Level	Non-Reactive @ Donor Level
HBsAg	Non-Reactive @ Donor Level	Non-Reactive @ Donor Level
HCV	Non-Reactive @ Donor Level	Non-Reactive @ Donor Level
RPR	Non-Reactive @ Donor Level	Non-Reactive @ Donor Level

Performance testing: Each level of product tested per below.

Level A

Test	Specification	Results	Actions
pH @ 25C (pH Meter)	6.75 - 8.15	7.7	Pass
Optical Density @ 700nm (Beckman Spectrophotometer)	< 2.0	0.012nm	Pass

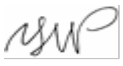
Level B

Test	Specification	Results	Actions
pH @ 25C (pH Meter)	7.00 -7.70	7.52	Pass
Optical Density @ 700nm (Beckman Spectrophotometer)	< 2.0	0.078nm	Pass

Level C

Test	Specification	Results	Actions
pH @ 25C (pH Meter)	7.00 -7.70	7.48	Pass
Optical Density @ 700nm (Beckman Spectrophotometer)	< 2.0	0.049nm	Pass

QC Ref: QCR- TMC-300-24-00 **Date: 2024-04-03**

QC Approval: 

Janet M Varona
Quality Control Supervisor

Monobind Inc.
100 North Pointe Drive
Lake Forest, California 92630 USA

ВЕКТОР

БЕСТ

Набор реагентов для иммуноферментного выявления иммуноглобулинов класса G к *Mycoplasma hominis*

ИНСТРУКЦИЯ ПО ПРИМЕНЕНИЮ

Утверждена 10.12.2014

Mycoplasma hominis – IgG – ИФА – БЕСТ

НАБОР РЕАГЕНТОВ

D-4352

1. НАЗНАЧЕНИЕ

1.1. Набор реагентов предназначен для выявления иммуноглобулинов класса G (IgG) к антигену p120 *Mycoplasma hominis* в сыворотке *(плазме)* крови человека и может быть использован в клинических и эпидемиологических исследованиях.

1.2. Набор реагентов рассчитан на проведение 96 анализов, включая контроли. Возможны 12 независимых постановок ИФА, при каждой из которых 3 лунки используют для постановки контролей.

2. ХАРАКТЕРИСТИКА НАБОРА

2.1. Принцип действия.

Метод определения основан на твёрдофазном иммуноферментном анализе с применением рекомбинантного антигена. Во время первой инкубации, при наличии в исследуемых образцах иммуноглобулинов класса G к *Mycoplasma hominis*, происходит их связывание с иммобилизованным на поверхности лунок планшета рекомбинантным антигеном p120 *Mycoplasma hominis*. Не связавшийся материал удаляют отмывкой.

На второй стадии антитела к IgG человека, меченные пероксидазой хрена *(конъюгат),* свя-

зываются с комплексом «антиген-антитело». Не связавшийся конъюгат удаляют отмывкой.

Во время третьей инкубации с раствором тетраметилбензидина происходит окрашивание раствора в лунках, содержащих комплексы «антиген-антитело».

Реакцию останавливают добавлением стоп-реагента. Результаты ИФА регистрируют с помощью спектрофотометра, измеряя **оптическую плотность (ОП)** в двухволновом режиме: основной фильтр – 450 нм, референс-фильтр – в диапазоне 620–650 нм. Допустима регистрация результатов только с фильтром 450 нм. Интенсивность жёлтого окрашивания пропорциональна количеству содержащихся в исследуемом образце иммуноглобулинов класса G к *Mycoplasma hominis*.

После измерения оптической плотности раствора в лунках на основании рассчитанного значения $ОП_{крит}$ анализируемые образцы оцениваются как положительные, сомнительные или отрицательные.

2.2. Состав набора:

- планшет разборный с иммобилизованным рекомбинантным антигеном p120 *Mycoplasma hominis* – 1 шт.;

- положительный контрольный образец ($К^+$), инактивированный – на основе инактивированной сыворотки крови человека, содержащий иммуноглобулины класса G к *Mycoplasma hominis* – прозрачная жидкость красного цвета – 1 фл., 0,5 мл;
- отрицательный контрольный образец ($К^-$), инактивированный – на основе инактивированной сыворотки крови человека, не содержащий иммуноглобулины класса G к *Mycoplasma hominis* – прозрачная жидкость светло-жёлтого цвета – 1 фл., 1 мл;
- конъюгат, концентрат – антитела к IgG человека, меченные пероксидазой хрена – прозрачная жидкость синего цвета – 1 фл., 1,5 мл;
- раствор для разведения конъюгата (РК) – бесцветная слегка опалесцирующая жидкость — 1 фл., 13 мл;
- разводящий буфер для сывороток (РБС) – прозрачная жидкость красного цвета – 1 фл., 13 мл;
- 25-кратный концентрат фосфатно-солевого буферного раствора с твином (ФСБ-Т×25) – прозрачная или слегка опалесцирующая бесцветная жидкость, возможно выпадение осадка солей, растворяющегося при нагревании – 1 фл., 28 мл;
- раствор ТМБ – прозрачная бесцветная или с желтоватым оттенком жидкость – 1 фл., 13 мл;
- стоп-реагент – прозрачная бесцветная жидкость – 1 фл., 12 мл.

Набор дополнительно комплектуется:

- плёнкой для заклеивания планшета – 3 шт.;
- ванночками для реагентов – 2 шт.;
- наконечниками для пипетки на 4–200 мкл – 16 шт.

3. АНАЛИТИЧЕСКИЕ И ДИАГНОСТИЧЕСКИЕ ХАРАКТЕРИСТИКИ

3.1. Результат качественного определения набором иммуноглобулинов класса G к *Mycoplasma hominis* должен соответствовать требованиям СПП *(рег. № 05-2-176),* включающей образцы сывороток, содержащие специфические IgG к *Mycoplasma hominis*: **чувствительность** по иммуноглобулинам класса G к *Mycoplasma hominis* – 100%.

3.2. Результат качественного определения набором иммуноглобулинов класса G к *Mycoplasma hominis* должен соответствовать требованиям СПП *(рег. № 05-2-176)*, включающей образцы сывороток, не содержащие IgG к *Mycoplasma hominis*: **специфичность** по иммуноглобулинам класса G к *Mycoplasma hominis* – 100%.

4. МЕРЫ ПРЕДОСТОРОЖНОСТИ

Потенциальный риск применения набора – класс 2а *(Приказ МЗ РФ от 06.06.2012 № 4н)*.

При работе с исследуемыми сыворотками и контрольными образцами следует соблюдать меры предосторожности, принятые при работе с потенциально инфекционным материалом:

* работать в резиновых перчатках;
* не пипетировать растворы ртом;
* все использованные материалы дезинфицировать в соответствии с требованиями с СП 1.3.2322-08 от 01.05.08 и МУ-287-113 от 30.12.98;
* утилизацию или уничтожение, дезинфекцию наборов реагентов следует проводить в соответствии с СанПиН 2.1.7.2790-10 «Санитарно-эпидемиологические требования к обращению с медицинскими отходами» и МУ-287-113 «Методические указания по дезинфекции, предстерилизационной очистке и стерилизации изделий медицинского назначения».

5. ОБОРУДОВАНИЕ И МАТЕРИАЛЫ

– Спектрофотометр, позволяющий проводить измерения оптической плотности растворов в лунках планшета при длине волны 450 нм и/или в двухволновом режиме при основной длине волны 450 нм и длине волны сравнения в диапазоне 620–650 нм;
– термостат, поддерживающий температуру (37±1) °С;
– холодильник бытовой;
– пипетки полуавтоматические одноканальные с переменным или фиксированным объёмом со сменными наконечниками, позволяющие отбирать объёмы жидкости от 5 до 1000 мкл;
– пипетка полуавтоматическая многоканальная со сменными наконечниками, позволяющая отбирать объёмы жидкостей от 5 до 300 мкл;
– промывочное устройство для планшета;
– перчатки медицинские диагностические одноразовые;
– бумага фильтровальная лабораторная;
– цилиндр мерный 2-го класса точности вместимостью 100 мл;
– цилиндр вместимостью 1000 мл;
– вода дистиллированная;
– дезинфицирующий раствор.

6. АНАЛИЗИРУЕМЫЕ ОБРАЗЦЫ

– Допускается использование образцов, хранившихся при температуре (2–8) °С не более 5 суток, либо при температуре минус (20±4) °С, если необходимо более длительное хранение.

– Сыворотки, содержащие взвешенные частицы, могут дать неправильный результат. Такие образцы перед использованием следует центрифугировать при 3000 об/мин в течение 10–15 минут.

– Нельзя использовать проросшие, гемолизированные, гиперлипидные сыворотки или подвергавшиеся многократному замораживанию и оттаиванию.

7. ПРОВЕДЕНИЕ ИММУНОФЕРМЕНТНОГО АНАЛИЗА

7.1. **ВНИМАНИЕ! Тщательное соблюдение описанных ниже требований позволит избежать искажения результатов ИФА.**

– Перед постановкой реакции все компоненты набора необходимо выдержать при температуре (18–25)°С не менее 30 минут.

– Для приготовления растворов и проведения ИФА следует использовать чистую мерную посуду и автоматические пипетки с погрешностью измерения объёмов не более 5%.

– После отбора необходимого количества стрипов оставшиеся сразу упаковать в пакет с осушителем. Упакованные стрипы, плотно закрытые флаконы с исходными компонентами хранить при (2–8) °С.

– Раствор конъюгата в рабочем разведении готовить непосредственно перед использованием.

– Раствор ТМБ готов для использования. Необходимо исключить воздействие прямого света на раствор ТМБ.

– При промывке лунки *(стрипа, планшета)* заполнять полностью, не допуская переливания промывочного раствора через края лунок, и не касаясь лунок наконечником пипетки.

Время между заполнением и опорожнением лунок должно быть не менее 30 секунд.

– При использовании автоматического или ручного промывателя необходимо следить за состоянием ёмкости для промывочного раствора и соединительных шлангов: в них не должно быть «заростов». Раз в неделю желательно ёмкость для промывочного раствора и шланги промывать 70% спиртом.
– Не допускать высыхания лунок планшета между отдельными операциями.
– При постановке ИФА нельзя использовать компоненты из наборов разных серий или смешивать их при приготовлении растворов, кроме неспецифических компонентов *(ФСБ-Т×25), раствор ТМБ, стоп-реагент)*, которые взаимозаменяемы в наборах АО «Вектор-Бест».
– При приготовлении растворов и проведении ИФА следует использовать одноразовые наконечники для дозаторов.
– Посуду *(ванночки)*, используемые для работы с растворами конъюгата и ТМБ, не обрабатывать дезинфицирующими растворами и моющими средствами.
– В случае повторного использования посуду *(ванночки)* для раствора конъюгата промыть

проточной водой и тщательно ополоснуть дистиллированной водой, посуду *(ванночки)* для раствора ТМБ сразу после работы необходимо промыть 50% раствором этилового спирта, а затем дистиллированной водой.

– Для дезинфекции посуды и материалов, контактирующих 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 *(четвертичных аммониевых соединений)*, спиртов, третичных аминов.
– Пипетки и рабочие поверхности обрабатывать только 70% раствором этилового спирта. Не использовать перекись водорода, хлорамин и т.д.

7.2. Приготовление реагентов.

7.2.1. Промывочный раствор.

Взболтать содержимое флакона с ФСБ-Т×25. При выпадении осадка солей в концентрате прогреть его перед разведением до полного растворения осадка.

В соответствии с числом используемых стрипов отобрать необходимое количество

Таблица расхода реагентов

	Количество используемых стрипов											
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Приготовление промывочного раствора												
ФСБ-Т×25, мл	2	4	6	8	10	12	14	16	18	20	22	24
Дистиллированная вода, мл	до 50	до 100	до 150	до 200	до 250	до 300	до 350	до 400	до 450	до 500	до 550	до 600
Приготовление раствора конъюгата в рабочем разведении												
Конъюгат (концентрат), мл	0,1	0,2	0,3	0,4	0,5	0,6	0,7	0,8	0,9	1,0	1,1	1,2
РК, мл	1,0	2,0	3,0	4,0	5,0	6,0	7,0	8,0	9,0	10,0	11,0	12,0
Раствор ТМБ												
Раствор ТМБ, мл	1,0	2,0	3,0	4,0	5,0	6,0	7,0	8,0	9,0	10,0	11,0	12,0

ФСБ-Т×25 *(см. таблицу)* и развести дистиллированной водой до указанного в таблице объёма или содержимое 1 флакона – до **700 мл.**

Хранение: при температуре (2–8) °С до 72 часов.

7.2.2. Контрольные образцы.

Контрольные образцы ($К^+$ и $К^-$) готовы к использованию.

Хранение: при температуре (2–8) °С в течение всего срока годности набора.

7.2.3. Раствор конъюгата в рабочем разведении.

Внимание! *Для работы с конъюгатом рекомендуем использовать* ***одноразовые*** *наконечники для пипеток.*

Внимание! *Раствор конъюгата в рабочем разведении готовить в пластиковой ванночке, входящей в состав набора, непосредственно перед использованием!*

Перед приготовлением раствора конъюгата в рабочем разведении необходимо аккуратно перемешать, не допуская вспенивания, содержимое флаконов с концентратом конъюгата и с РК.

В пластиковую ванночку внести необходимое количество РК, добавить соответствующее

количество конъюгата *(см. таблицу)* и аккуратно перемешать пипетированием до получения равномерного окрашивания.

7.2.4. Раствор ТМБ.

Внимание! *Раствор ТМБ готов к использованию. Необходимо исключить воздействие света на раствор ТМБ.*

Непосредственно перед использованием отобрать в пластиковую ванночку **только** необходимое в соответствии с числом используемых стрипов количество раствора ТМБ *(см. таблицу)*. Остатки раствора ТМБ из ванночки утилизировать *(не сливать во флакон с исходным раствором ТМБ)*.

Хранение: при температуре (2–8) °С в течение всего срока годности набора.

7.3. Проведение анализа.

7.3.1. Подготовить необходимое количество стрипов к работе. Оставшиеся – сразу упаковать во избежание губительного воздействия влаги. Для этого стрипы поместить в цефленовый пакет с влагопоглотителем, тщательно закрыть пакет пластиковой застёжкой. Упакованные таким образом стрипы хранить при (2–8) °С до конца срока годности набора.

Приготовить промывочный раствор *(п. 7.2.1)*, контрольные образцы *(п. 7.2.2)*.

7.3.2. Перед постановкой ИФА лунки стрипов промыть один раз промывочным раствором, заливая в каждую лунку по 400 мкл промывочного раствора. По истечении 5 минут раствор аккуратно удалить в сосуд с дезинфицирующим раствором.

По окончании промывки необходимо тщательно удалить влагу из лунок, постукивая перевёрнутыми стрипами по сложенной в несколько слоёв фильтровальной бумаге. *Не допускать высыхания лунок стрипов между отдельными операциями при постановке реакции.*

7.3.3. Во все лунки стрипов внести по **80 мкл РБС**. В одну лунку внести **20 мкл К$^+$**, в две другие лунки по **20 мкл К$^-$**, в остальные лунки – по **20 мкл исследуемых образцов**, получая таким образом, разведение 1:5. Внесение образцов должно сопровождаться аккуратным перемешиванием *(пипетирование не менее 4 раз)*. Не допускать вспенивания и касания наконечником дна и стенок лунки.

Лунки заклеить плёнкой и инкубировать при температуре (37±1) °С **30 минут**.

За 5 минут до окончания инкубации приготовить раствор конъюгата в рабочем разведении.

7.3.4. По окончании инкубации содержимое лунок собрать в сосуд с дезинфицирующим раствором, промыть лунки стрипов 5 раз промывочным раствором и тщательно удалить влагу.

Внимание! *Каждую лунку при промывке необходимо заполнять полностью (400 мкл промывочного раствора). Необходимо добиваться полного опорожнения лунок после каждого их заполнения. Время между заполнением и опорожнением лунок должно быть не менее 30 сек.*

7.3.5. Во все лунки планшета внести по **100 мкл раствора конъюгата в рабочем разведении.**

Внимание! *Для внесения раствора конъюгата в рабочем разведении использовать пластиковую ванночку и* ***одноразовые*** *наконечники, входящие в состав набора.*

Заклеить лунки плёнкой и инкубировать при температуре (37±1) °С **30 минут**.

По окончании инкубации содержимое лунок собрать в сосуд с дезинфицирующим раствором, лунки промыть 5 раз промывочным раствором и удалить влагу, как описано выше.

7.3.6. Во все лунки внести по **100 мкл раствора ТМБ**.

Внимание! *Для внесения раствора ТМБ использовать пластиковую ванночку и* ***одноразовые*** *наконечники, входящие в состав набора.*

Стрипы поместить в защищённое от света место при температуре (18–25) °С на **30 минут.**

7.3.7. Остановить реакцию добавлением во все лунки по **100 мкл стоп-реагента** и через 2–3 минуты измерить ОП.

Следует избегать попадания стоп-реагента на одежду и открытые участки тела. При попадании – промыть большим количеством воды.

8. РЕГИСТРАЦИЯ РЕЗУЛЬТАТОВ

Результаты ИФА регистрировать с помощью спектрофотометра, измеряя ОП в двухволновом режиме: основной фильтр – 450 нм, референс-фильтр – в диапазоне 620–650 нм. Допустима регистрация результатов только с фильтром 450 нм.

Выведение спектрофотометра на нулевой уровень *(«бланк»)* осуществлять по воздуху.

9. УЧЁТ РЕЗУЛЬТАТОВ РЕАКЦИИ

9.1. Результаты исследований учитывать только при соблюдении следующих условий:

– среднее значение ОП в лунках с отрицательным контрольным образцом не более 0,25 ($ОП_{ср}К^{-} \leq 0{,}25$).

– значение ОП в лунке с положительным контрольным образцом не менее 0,6 ($ОПК^{+} \geq 0{,}60$).

Вычислить **критическое значение оптической плотности ($ОП_{крит}$)** по формуле:

$$\mathbf{ОП_{крит} = ОП_{ср}(К^{-}) + 0{,}3},$$

где $ОП_{ср}(К^{-})$ — среднее значение ОП для отрицательного контрольного образца.

Исследуемый образец оценить как:

– **отрицательный**, т.е. не содержащий IgG к *Mycoplasma hominis*, если полученное для него значение $ОП_{обр} \leq ОП_{крит} - 0{,}05$;

– **положительный,** т.е. содержащий IgG к *Mycoplasma hominis*, если значение $ОП_{обр} \geq ОП_{крит} + 0{,}05$;

– **сомнительный**, если $ОП_{крит} - 0{,}05 < ОП_{обр} < ОП_{крит} + 0{,}05$.

9.2. Интерпретация результатов.

ОП образца	Результат	Титр IgG
от 0 до (ОП$_{крит}$ – 0,05)	отрицательный	–
от (ОП$_{крит}$ – 0,05) до (ОП$_{крит}$ + 0,05)	сомнительный	–
от (ОП$_{крит}$ + 0,05) до 2 × ОП$_{крит}$	слабоположительный	1:5
от 2 × ОП$_{крит}$ до 4 × ОП$_{крит}$	положительный	1:10
от 4 × ОП$_{крит}$ до 8 × ОП$_{крит}$	сильноположительный	1:20
от 8 × ОП$_{крит}$ до 11 × ОП$_{крит}$	сильноположительный	1:40
более 11 × ОП$_{крит}$	сильноположительный	1:80

Пациентам с сомнительными и положительными результатами рекомендуется дополнительное обследование *(выявление возбудителя, обследование парных сывороток).* Все клинические и лабораторные данные должны быть рассмотрены в совокупности.

10. УСЛОВИЯ ХРАНЕНИЯ И ЭКСПЛУАТАЦИИ НАБОРА

10.1. Транспортирование набора должно проводиться при температуре (2–8) °С. Допускается транспортирование при температуре до 25 °С не более 10 суток.

Замораживание не допускается.

10.2. Хранение набора в упаковке предприятия-изготовителя должно производиться при температуре (2–8) °С.

Замораживание не допускается.

10.3. Срок годности набора реагентов – 12 месяцев со дня выпуска.

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 счёт 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 ***«Mycoplasma hominis-IgG-ИФА-БЕСТ»****, обращаться в* ***АО «Вектор-Бест»*** *по адресу:*
630117, г. Новосибирск-117, а/я 492,
тел.: (383) 332-92-49, 227-60-30;
тел./факс: (383) 332-94-47, 332-94-44;
E-mail: plkobtk@vector-best.ru

ДОПОЛНИТЕЛЬНАЯ ИНФОРМАЦИЯ ДЛЯ ПОТРЕБИТЕЛЕЙ:

– Набор реагентов предназначен для профессионального применения и должен использоваться обученным персоналом;

– 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 следует проводить по МУ-287-113 «Методические указания по дезинфекции, предстерилизационной очистке и стерилизации изделий медицинского назначения»;

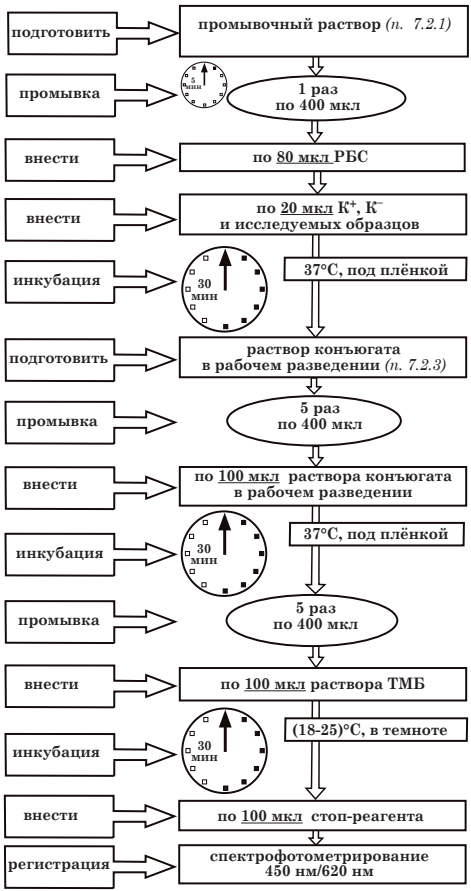
– Требования безопасности к медицинским лабораториям приведены в ГОСТ Р 52905-2007;

– Не применять набор реагентов по назначению после окончания срока годности;

– Транспортирование должно проводиться всеми видами крытого транспорта в соответствии с правилами перевозок, действующими на транспорте данного вида.

– Производитель гарантирует соответствие выпускаемых изделий требованиям нормативной и технической документации.

Схема анализа D-4352



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
	Дата изготовления: XXXX-XX-XX Формат даты: год-месяц-число		Изготовитель
	Использовать до: XXXX-XX-XX Формат даты: год-месяц-число		Обратитесь к Инструкции по применению
	Осторожно! Обратитесь к Инструкции по применению		

К онсультацию специалиста по работе с набором можно получить по тел.: (383) 332-81-44.

18.04.16

АКЦИОНЕРНОЕ ОБЩЕСТВО
«ВЕКТОР-БЕСТ»

Международный сертификат
ISO 13485

Наш адрес: 630117, Новосибирск-117, а/я 492

Тел.: (383) 332-37-58, 332-37-10, 332-36-34,
332-67-49, 332-67-52

Тел./факс: (383) 227-73-60 (многоканальный)

E-mail: vbmarket@vector-best.ru
Internet: www.vector-best.ru

ВЕКТОР БЕСТ

Набор реагентов для иммуноферментного выявления иммуноглобулинов класса А к *Mycoplasma hominis*

ИНСТРУКЦИЯ ПО ПРИМЕНЕНИЮ

Утверждена 10.12.14

Mycoplasma hominis – IgA – ИФА – БЕСТ

НАБОР РЕАГЕНТОВ **D-4358**

1. НАЗНАЧЕНИЕ

1.1. Набор реагентов предназначен для выявления иммуноглобулинов класса А (IgA) к антигену p120 *Mycoplasma hominis* в сыворотке *(плазме)* крови человека и может быть использован в клинических и эпидемиологических исследованиях.

1.2. Набор реагентов рассчитан на проведение 96 анализов, включая контроли. Возможны 12 независимых постановок ИФА, при каждой из которых 3 лунки используют для постановки контролей.

2. ХАРАКТЕРИСТИКА НАБОРА

2.1. Принцип действия.

Метод определения основан на твёрдофазном иммуноферментном анализе с применением рекомбинантного антигена. Во время первой инкубации, при наличии в исследуемых образцах иммуноглобулинов класса А к *Mycoplasma hominis*, происходит их связывание с иммобилизованным на поверхности лунок планшета рекомбинантным антигеном p120 *Mycoplasma hominis*. Не связавшийся материал удаляют отмывкой.

На второй стадии антитела к IgA человека, меченные пероксидазой хрена *(конъюгат),* свя-

зываются с комплексом «антиген-антитело». Не связавшийся конъюгат удаляют отмывкой.

Во время третьей инкубации с раствором тетраметилбензидина происходит окрашивание раствора в лунках, содержащих комплексы «антиген-антитело».

Реакцию останавливают добавлением стоп-реагента. Результаты ИФА регистрируют с помощью спектрофотометра, измеряя **оптическую плотность (ОП)** в двухволновом режиме: основной фильтр – 450 нм, референс-фильтр – в диапазоне 620–650 нм. Допустима регистрация результатов только с фильтром 450 нм. Интенсивность жёлтого окрашивания пропорциональна количеству содержащихся в исследуемом образце иммуноглобулинов класса А к *Mycoplasma hominis*.

После измерения оптической плотности раствора в лунках на основании рассчитанного значения $ОП_{крит}$ анализируемые образцы оцениваются как положительные, сомнительные или отрицательные.

2.2. Состав набора:

- планшет разборный с иммобилизованным рекомбинантным антигеном p120 *Mycoplasma hominis* – 1 шт.;

- положительный контрольный образец ($К^+$), инактивированный – на основе инактивированной сыворотки крови человека, содержащий иммуноглобулины класса А к *Mycoplasma hominis* – прозрачная жидкость красного цвета – 1 фл., 0,5 мл;
- отрицательный контрольный образец ($К^–$), инактивированный – на основе инактивированной сыворотки крови человека, не содержащий иммуноглобулины класса А к *Mycoplasma hominis* – прозрачная жидкость светло-жёлтого цвета – 1 фл., 1 мл;
- конъюгат, концентрат – антитела к IgA человека, меченные пероксидазой хрена – прозрачная жидкость синего цвета – 1 фл., 1,5 мл;
- раствор для разведения конъюгата (РК) – бесцветная слегка опалесцирующая жидкость –1 фл., 13 мл;
- раствор для разведения сывороток (РС) – прозрачная жидкость жёлто-красного цвета – 1 фл., 13 мл;
- 25-кратный концентрат фосфатно-солевого буферного раствора с твином (ФСБ-Т×25) – прозрачная или слегка опалесцирующая бесцветная жидкость, возможно выпадение осадка солей, растворяющегося при нагревании – 1 фл., 28 мл;
- раствор ТМБ – прозрачная бесцветная или с желтоватым оттенком жидкость — 1 фл., 13 мл;
- стоп-реагент – прозрачная бесцветная жидкость – 1 фл., 12 мл.

Набор дополнительно комплектуется:

- плёнкой для заклеивания планшета – 3 шт.;
- ванночками для реагентов – 2 шт.;
- наконечниками для пипетки на 4–200 мкл – 16 шт.

3. АНАЛИТИЧЕСКИЕ И ДИАГНОСТИЧЕСКИЕ ХАРАКТЕРИСТИКИ

3.1. Результат качественного определения набором иммуноглобулинов класса А к *Mycoplasma hominis* должен соответствовать требованиям СПП *(рег. № 05-2-178),* включающей образцы сывороток, содержащие специфические IgA к *Mycoplasma hominis*: **чувствительность** по иммуноглобулинам класса А к *Mycoplasma hominis* – 100%.

3.2. Результат качественного определения набором иммуноглобулинов класса А к *Mycoplasma hominis* должен соответствовать требованиям СПП *(рег. № 05-2-178)*, включающей образцы сывороток, не содержащие IgA к *Mycoplasma hominis*: **специфичность** по иммуноглобулинам класса А к *Mycoplasma hominis* – 100%.

4. МЕРЫ ПРЕДОСТОРОЖНОСТИ

Потенциальный риск применения набора – класс 2а *(Приказ МЗ РФ от 06.06.2012 № 4н)*.

При работе с исследуемыми сыворотками и контрольными образцами следует соблюдать меры предосторожности, принятые при работе с потенциально инфекционным материалом:

* работать в резиновых перчатках;
* не пипетировать растворы ртом;
* все использованные материалы дезинфицировать в соответствии с требованиями с СП 1.3.2322-08 от 01.05.08 и МУ-287-113 от 30.12.98;
* утилизацию или уничтожение, дезинфекцию наборов реагентов следует проводить в соответствии с СанПиН 2.1.7.2790-10 «Санитарно-эпидемиологические требования к обращению с медицинскими отходами» и МУ-287-113 «Методические указания по дезинфекции, предстерилизационной очистке и стерилизации изделий медицинского назначения».

5. ОБОРУДОВАНИЕ И МАТЕРИАЛЫ

– Спектрофотометр, позволяющий проводить измерения оптической плотности растворов в лунках планшета при длине волны 450 нм и/или в двухволновом режиме при основной длине волны 450 нм и длине волны сравнения в диапазоне 620–650 нм;
– термостат, поддерживающий температуру (37±1) °С;
– холодильник бытовой;
– пипетки полуавтоматические одноканальные с переменным или фиксированным объёмом со сменными наконечниками, позволяющие отбирать объёмы жидкости от 5 до 1000 мкл;
– пипетка полуавтоматическая многоканальная со сменными наконечниками, позволяющая отбирать объёмы жидкостей от 5 до 300 мкл;
– промывочное устройство для планшета;
– перчатки медицинские диагностические одноразовые;
– бумага фильтровальная лабораторная;
– цилиндр мерный 2-го класса точности вместимостью 100 мл;
– цилиндр вместимостью 1000 мл;
– вода дистиллированная;
– дезинфицирующий раствор.

6. АНАЛИЗИРУЕМЫЕ ОБРАЗЦЫ

– Допускается использование образцов, хранившихся при температуре (2–8) °С не более 5 суток, либо при температуре минус (20±4) °С, если необходимо более длительное хранение.

– Сыворотки, содержащие взвешенные частицы, могут дать неправильный результат. Такие образцы перед использованием следует центрифугировать при 3000 об/мин в течение 10–15 минут.

– Нельзя использовать проросшие, гемолизированные, гиперлипидные сыворотки или подвергавшиеся многократному замораживанию и оттаиванию.

7. ПРОВЕДЕНИЕ ИММУНОФЕРМЕНТНОГО АНАЛИЗА

7.1. **ВНИМАНИЕ! Тщательное соблюдение описанных ниже требований позволит избежать искажения результатов ИФА.**

– Перед постановкой реакции все компоненты набора необходимо выдержать при температуре (18–25)°С не менее 30 минут.

– Для приготовления растворов и проведения ИФА следует использовать чистую мерную посуду и автоматические пипетки с погрешностью измерения объёмов не более 5%.

– После отбора необходимого количества стрипов оставшиеся сразу упаковать в пакет с осушителем. Упакованные стрипы, плотно закрытые флаконы с исходными компонентами хранить при (2–8) °С.
– Раствор конъюгата в рабочем разведении готовить непосредственно перед использованием.
– Раствор ТМБ готов для использования. Необходимо исключить воздействие прямого света на раствор ТМБ.
– При промывке лунки *(стрипа, планшета)* заполнять полностью, не допуская переливания промывочного раствора через края лунок, и не касаясь лунок наконечником пипетки. Время между заполнением и опорожнением лунок должно быть не менее 30 секунд.
– При использовании автоматического или ручного промывателя необходимо следить за состоянием ёмкости для промывочного раствора и соединительных шлангов: в них не должно быть «заростов». Раз в неделю желательно ёмкость для промывочного раствора и шланги промывать 70% спиртом.
– Не допускать высыхания лунок планшета между отдельными операциями.
– При постановке ИФА нельзя использовать компоненты из наборов разных серий или

смешивать их при приготовлении растворов, кроме неспецифических компонентов *(ФСБ-Т×25), раствор ТМБ, стоп-реагент)*, которые взаимозаменяемы в наборах АО «Вектор-Бест».

– При приготовлении растворов и проведении ИФА следует использовать **одноразовые** наконечники для дозаторов.

– Посуду *(ванночки)*, используемые для работы с растворами конъюгата и ТМБ, не обрабатывать дезинфицирующими растворами и моющими средствами.

– В случае повторного использования посуду *(ванночки)* для раствора конъюгата промыть проточной водой и тщательно ополоснуть дистиллированной водой, посуду *(ванночки)* для раствора ТМБ сразу после работы необходимо промыть 50% раствором этилового спирта, а затем дистиллированной водой.

– Для дезинфекции посуды и материалов, контактирующих 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 *(четвертичных аммониевых соединений)*, спиртов, третичных аминов.

– Пипетки и рабочие поверхности обрабатывать только 70% раствором этилового спирта. Не использовать перекись водорода, хлорамин и т.д.

7.2. Приготовление реагентов.

7.2.1. Промывочный раствор.

Взболтать содержимое флакона с ФСБ-Т×25. При выпадении осадка солей в концентрате прогреть его перед разведением до полного растворения осадка.

В соответствии с числом используемых стрипов отобрать необходимое количество ФСБ-Т×25 *(см. таблицу)* и развести дистиллированной водой до указанного в таблице объёма или содержимое 1 флакона – до **700 мл.**

Хранение: при температуре (2–8) °С до 72 часов.

7.2.2. Контрольные образцы.

Контрольные образцы ($К^+$ и $К^-$) готовы к использованию.

Хранение: при температуре (2–8) °С в течение всего срока годности набора.

7.2.3. Раствор конъюгата в рабочем разведении.

Внимание! *Для работы с конъюгатом рекомендуем использовать* ***одноразовые*** *наконечники для пипеток.*

Таблица расхода реагентов

	Количество используемых стрипов											
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Приготовление промывочного раствора												
ФСБ-Т×25, мл	2	4	6	8	10	12	14	16	18	20	22	24
Дистиллированная вода, мл	до 50	до 100	до 150	до 200	до 250	до 300	до 350	до 400	до 450	до 500	до 550	до 600
Приготовление раствора конъюгата в рабочем разведении												
Конъюгат (концентрат), мл	0,1	0,2	0,3	0,4	0,5	0,6	0,7	0,8	0,9	1,0	1,1	1,2
РК, мл	1,0	2,0	3,0	4,0	5,0	6,0	7,0	8,0	9,0	10,0	11,0	12,0
Раствор ТМБ												
Раствор ТМБ, мл	1,0	2,0	3,0	4,0	5,0	6,0	7,0	8,0	9,0	10,0	11,0	12,0

Внимание! *Раствор конъюгата в рабочем разведении готовить в пластиковой ванночке, входящей в состав набора, непосредственно перед использованием!*

Перед приготовлением раствора конъюгата в рабочем разведении необходимо аккуратно перемешать, не допуская вспенивания, содержимое флаконов с концентратом конъюгата и с РК.

В пластиковую ванночку внести необходимое количество РК, добавить соответствующее количество конъюгата *(см. таблицу)* и аккуратно перемешать пипетированием до получения равномерного окрашивания.

7.2.4. Раствор ТМБ.

Внимание! *Раствор ТМБ готов к использованию. Необходимо исключить воздействие света на раствор ТМБ.*

Непосредственно перед использованием отобрать в пластиковую ванночку **только** необходимое в соответствии с числом используемых стрипов количество раствора ТМБ *(см. таблицу)*. Остатки раствора ТМБ из ванночки утилизировать *(не сливать во флакон с исходным раствором ТМБ)*.

Хранение: при температуре (2–8) °С в течение всего срока годности набора.

7.3. Проведение анализа.

7.3.1. Подготовить необходимое количество стрипов к работе. Оставшиеся – сразу упаковать во избежание губительного воздействия влаги. Для этого стрипы поместить в цефленовый пакет с влагопоглотителем, тщательно закрыть пакет пластиковой застёжкой. Упакованные таким образом стрипы хранить при (2–8) °С до конца срока годности набора.

Приготовить промывочный раствор *(п. 7.2.1)*, контрольные образцы *(п. 7.2.2)*.

7.3.2. Перед постановкой ИФА лунки стрипов промыть один раз промывочным раствором, заливая в каждую лунку по 400 мкл промывочного раствора. По истечении 5 мин раствор аккуратно удалить в сосуд с дезинфицирующим раствором.

По окончании промывки необходимо тщательно удалить влагу из лунок, постукивая перевёрнутыми стрипами по сложенной в несколько слоёв фильтровальной бумаге. *Не допускать высыхания лунок стрипов между отдельными операциями при постановке реакции.*

7.3.3. Во все лунки стрипов внести по **80 мкл РС**. В одну лунку внести **20 мкл К$^+$**, в две другие лунки по **20 мкл К$^-$**, в остальные лунки – по **20 мкл исследуемых образцов**, получая таким образом, разведение 1:5. Внесение образцов

должно сопровождаться аккуратным перемешиванием *(пипетирование не менее 4 раз)*. Не допускать вспенивания и касания наконечником дна и стенок лунки.

Лунки заклеить плёнкой и инкубировать при температуре (37±1) °С **30 минут**.

За 5 мин до окончания инкубации приготовить раствор конъюгата в рабочем разведении.

7.3.4. По окончании инкубации содержимое лунок собрать в сосуд с дезинфицирующим раствором, промыть лунки стрипов 5 раз промывочным раствором и тщательно удалить влагу.

Внимание! *Каждую лунку при промывке необходимо заполнять полностью (400 мкл промывочного раствора). Необходимо добиваться полного опорожнения лунок после каждого их заполнения. Время между заполнением и опорожнением лунок должно быть не менее 30 секунд.*

7.3.5. Во все лунки планшета внести по **100 мкл раствора конъюгата в рабочем разведении.**

Внимание! *Для внесения раствора конъюгата в рабочем разведении использовать пластиковую ванночку и* ***одноразовые*** *наконечники, входящие в состав набора.*

Заклеить лунки плёнкой и инкубировать при температуре (37±1) °С **30 минут**.

По окончании инкубации содержимое лунок собрать в сосуд с дезинфицирующим раствором, лунки промыть 5 раз промывочным раствором и удалить влагу, как описано выше.

7.3.6. Во все лунки внести по **100 мкл раствора ТМБ**.

Внимание! *Для внесения раствора ТМБ использовать пластиковую ванночку и* ***одноразовые*** *наконечники, входящие в состав набора.*

Стрипы поместить в защищённое от света место при температуре (18–25) °С на **30 минут.**

7.3.7. Остановить реакцию добавлением во все лунки по **100 мкл стоп-реагента** и через 2–3 минуты измерить ОП.

Следует избегать попадания стоп-реагента на одежду и открытые участки тела. При попадании – промыть большим количеством воды.

8. РЕГИСТРАЦИЯ РЕЗУЛЬТАТОВ

Результаты ИФА регистрировать с помощью спектрофотометра, измеряя ОП в двухволновом режиме: основной фильтр – 450 нм, референс-фильтр – в диапазоне 620–650 нм. Допустима регистрация результатов только с фильтром 450 нм.

Выведение спектрофотометра на нулевой уровень *(«бланк»)* осуществлять по воздуху.

9. УЧЁТ РЕЗУЛЬТАТОВ РЕАКЦИИ

9.1. Результаты исследований учитывать только при соблюдении следующих условий:

– среднее значение ОП в лунках с отрицательным контрольным образцом не более 0,25 ($ОП_{ср}К^{-} \leq 0{,}25$).

– значение ОП в лунке с положительным контрольным образцом не менее 0,6 ($ОПК^{+} \geq 0{,}60$).

Вычислить **критическое значение оптической плотности ($ОП_{крит}$)** по формуле:

$$\mathbf{ОП_{крит} = ОП_{ср}(К^{-}) + 0{,}3},$$

где $ОП_{ср}(К^{-})$ — среднее значение ОП для отрицательного контрольного образца.

Исследуемый образец оценить как:

– **отрицательный**, т.е. не содержащий IgA к *Mycoplasma hominis*, если полученное для него значение $ОП_{обр} \leq ОП_{крит} - 0{,}05$;

– **положительный,** т.е. содержащий IgA к *Mycoplasma hominis*, если значение $ОП_{обр} \geq ОП_{крит} + 0{,}05$;

– **сомнительный**, если $ОП_{крит} - 0{,}05 < ОП_{обр} < ОП_{крит} + 0{,}05$.

10. УСЛОВИЯ ХРАНЕНИЯ И ЭКСПЛУАТАЦИИ НАБОРА

10.1. Транспортирование набора должно проводиться при температуре (2–8) °С. Допускается транспортирование при температуре до 25 °С не более 10 суток. Замораживание не допускается.

10.2. Хранение набора в упаковке предприятия-изготовителя должно производиться при температуре (2–8) °С. Замораживание не допускается.

10.3. Срок годности набора реагентов – 12 месяцев со дня выпуска.

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 счёт заменить изделие, технические и функциональ-

ные 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 ***«Mycoplasma hominis-IgA-ИФА-БЕСТ»****, обращаться в* ***АО «Вектор-Бест»*** *по адресу:*
630117, г. Новосибирск-117, а/я 492,
тел.: (383) 332-92-49, 227-60-30;
тел./факс: (383) 332-94-47, 332-94-44;
E-mail: plkobtk@vector-best.ru

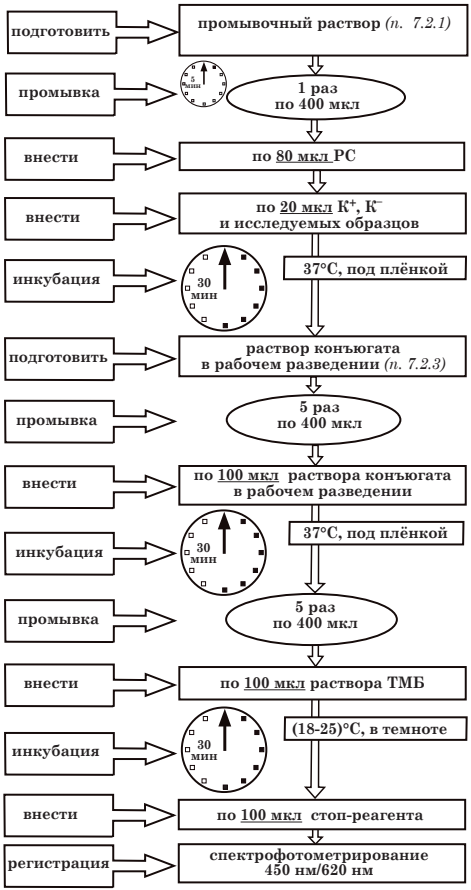
ДОПОЛНИТЕЛЬНАЯ ИНФОРМАЦИЯ ДЛЯ ПОТРЕБИТЕЛЕЙ:

– Набор реагентов предназначен для профессионального применения и должен использоваться обученным персоналом;
– 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 следует проводить по

МУ-287-113 «Методические указания по дезинфекции, предстерилизационной очистке и стерилизации изделий медицинского назначения»;

– Требования безопасности к медицинским лабораториям приведены в ГОСТ Р 52905-2007;
– Не применять набор реагентов по назначению после окончания срока годности;
– Транспортирование должно проводиться всеми видами крытого транспорта в соответствии с правилами перевозок, действующими на транспорте данного вида.
– Производитель гарантирует соответствие выпускаемых изделий требованиям нормативной и технической документации.

Схема анализа D-4358



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
	Дата изготовления: XXXX-XX-XX Формат даты: год-месяц-число		Изготовитель
	Использовать до: XXXX-XX-XX Формат даты: год-месяц-число		Обратитесь к Инструкции по применению
	Осторожно! Обратитесь к Инструкции по применению		

Консультацию специалиста по работе с набором можно получить по тел.: (383) 332-81-44.

18.04.16

АКЦИОНЕРНОЕ ОБЩЕСТВО
«ВЕКТОР-БЕСТ»

Международный сертификат
ISO 13485

Наш адрес: 630117, Новосибирск-117, а/я 492

Тел.: (383) 332-37-58, 332-37-10, 332-36-34,
332-67-49, 332-67-52

Тел./факс: (383) 227-73-60 (многоканальный)

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Internet: www.vector-best.ru