Monobind Inc.
Lake Forest, CA 92630, USA

AccuBind
ELISA Microwells

Total Triiodothyronine (T3) Test System
Product Code: 125-300

1.0 INTRODUCTION

Intended Use: The Quantitative Determination of Total Triiodothyronine Concentration in T3 Calibrators or Plasma by a Microplate Enzyme Immunoassay

2.0 SUMMARY AND EXPLANATION OF THE TEST

Measurement of serum triiodothyronine concentration is generally regarded as a valuable tool in the diagnosis of thyroid dysfunction. This importance has provided the impetus for the significant improvement in assay methodology that has occurred in the last two decades. The advent of monospecific antiserum and the discovery of blocking agents to the T3 binding serum proteins have enabled the development of procedurally simple radioimmunoassays (1,2).
This microplate enzyme immunoassay methodology provides the technician with optimum sensitivity while requiring few technical manipulations. In this method, serum reference, patient specimen, or control is first added to a microplate well. Enzyme-T3 conjugate is added, and then the reactants are mixed. A competition reaction results between the enzyme conjugate and the native triiodothyronine for a limited number of antibody combining sites immobilized on the well.
After the completion of the required incubation period, the antibody bound T3-enzyme conjugate is separated from the unbound T3-enzyme 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 triiodothyronine 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 T3 concentration.

3.0 PRINCIPLE

Competitive Enzyme Immunoassay (TYPE 5):
The essential reagents required for a solid phase enzyme immunoassay include immobilized antibody, enzyme-antigen conjugate and native antigen.
Upon mixing immobilized 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 insolubulized binding sites.

The interaction is illustrated by the following equation:

$$Enz_{Ag} + Ag + Ab_{C.W.} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} AgAb_{C.W.} + Enz_{Ag}Ab_{C.W.}$$

$Ab_{C.W.}$ = Monospecific Immobilized Antibody (Constant Quantity)
Ag = Native Antigen (Variable Quantity)
Enz_{Ag} = Enzyme-antigen Conjugate (Constant Quantity)
$AgAb_{C.W.}$ = Antigen-Antibody Complex
$Enz_{Ag}Ab_{C.W.}$ = Enzyme-antigen Conjugate -Antibody Complex
k_a = Rate Constant of Association
k_{-a} = Rate Constant of Disassociation
$K = k_a / k_{-a}$ = Equilibrium Constant

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 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. T3 Calibrators References – 1ml/vial - Icons A-F
Six (6) vials of serum reference for triiodothyronine at concentrations of 0 (**A**), 0.5 (**B**), 1.0 (**C**), 2.5 (**D**), 5.0(**E**) and 7.5(**F**) ng/ml. Store at 2-8°C. A preservative has been added. **For SI units: ng/ml x 1.536 = nmol/L**

B. T3 Enzyme Reagent – 1.5ml/vial - Icon Ⓔ
One (1) vial of T3-horseradish peroxidase (HRP) conjugate in an albumin-stabilizing matrix. A preservative has been added. Store at 2-8°C

C. T3/T4 Conjugate Buffer – 13ml - Icon Ⓑ
One (1) bottle reagent containing buffer, red dye, preservative, and binding protein inhibitors. Store at 2-8°C.

D. T3 Antibody Coated Plate – 96 wells - Icon
One 96-well microplate coated with Sheep anti-T3 serum and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

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

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

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

H. Stop Solution – 8ml/vial - Icon STOP
One (1) bottle of stop solution containing a strong acid (1N HCL). Store at 2-30°C.

I. Product Instructions.

Note 1: Do not use reagents beyond the kit expiration date.
Note 2: Opened reagents are stable for sixty (60) days when stored at 2-8°C. **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 96-well microplate. For other kit configurations, see table at end of IFU.

4.1 Materials Required But Not Provided:
1 Pipettes capable of delivering 50µl volumes with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100ml and 0.350ml volumes with a precision of better than 1.5%.
3. Adjustable volume (20-200µl) and (200-1000µl) dispenser(s) for conjugate and substrate preparation.
4. Microplate washers or a squeeze bottle (optional).
5. Microplate Reader with 450nm and 620-630nm wavelength absorbance capability.
6. Test tubes for preparation of enzyme conjugate and substrate A plus B.
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 T3 Calibrators products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Edition, 1988, HHS Publication No. (CDC) 88-8395.

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

6.0 SPECIMEN COLLECTION AND PREPARATION

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

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 of the specimen is required.

7.0 QUALITY CONTROL

Each laboratory should assay external controls at levels in the hypothyroid, euthyroid and hyperthyroid 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. Working Reagent A - T3-enzyme Conjugate Solution
Dilute the T3-enzyme conjugate 1:11 with T3/T4 conjugate buffer in a suitable container. For example, dilute 160µl of conjugate with 1.6ml of buffer for 16 wells (A slight excess of solution is made). This reagent should be used within twenty-four hours for maximum performance of the assay. Store at 2-8°C.
General Formula:
Amount of Buffer required = Number of wells ∗ 0.1
Quantity of T3-Enzyme necessary = # of wells * 0.01
i.e. = 16 x 0.1 = 1.6ml for Total T3/T4 Conjugate Buffer
16 x 0.01 = 0.16ml (160µl) for T3 enzyme conjugate

2. 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.

3. Working Substrate Solution
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 references 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 2-8ºC.**
2. Pipette 0.050 ml (50µl) of the appropriate serum reference, control or specimen into the assigned well.
3. Add 0.100 ml (100µl) of Working Reagent A, T3 Enzyme Reagent to all wells (see Reagent Preparation Section).
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. 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.**

Note: For re-assaying specimens with concentrations greater than 7.5ng/ml, pipette 25µl of the specimen and 25µl of the 0 serum reference into the sample well (this maintains a uniform protein concentration). Multiply the readout value by 2 to obtain the triiodothyronine concentration.

10.0 CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of triiodothyronine 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 T3 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 T3 for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis (y-axis) of the graph, find the intersecting point on the curve, and read the concentration (in ng/ml) from the horizontal axis (X-axis) of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance (1.130) intersects the dose response curve at 1.95ng/ml T3 concentration (See Figure 1).

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

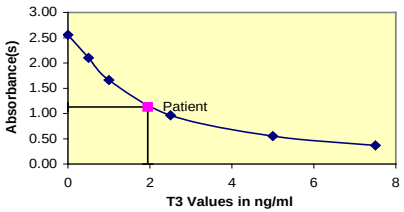
software is utilized, the validation of the software should be ascertained.

EXAMPLE 1

Sample I.D.	Well Number	Abs (A)	Mean Abs (B)	Value (ng/ml)
Cal A	A1	2.604	2.556	0
	B1	2.507		
Cal B	C1	2.073	2.101	0.5
	D1	2.128		
Cal C	E1	1.678	1.662	1.0
	F1	1.646		
Cal D	G1	0.964	0.966	2.5
	H1	0.969		
Cal E	A2	0.550	0.551	5.0
	B2	0.551		
Cal F	C2	0.372	0.370	7.5
	D2	0.369		
Ctrl 1	E2	1.701	1.726	***0.92***
	F2	1.638		
Ctrl 2	G2	0.755	0.734	***3.58***
	H2	0.791		
Patient	A3	1.145	1.130	***1.95***
	B3	1.115		

*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 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 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. Patient specimens with T3 concentrations above 7.5 ng/mL may be diluted ½ with '0' serum reference. The sample's concentration is obtained by multiplying the result by the dilution factor, 2.
10. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from Monobind's IFU may yield inaccurate results.
11. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.
12. It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.
13. Risk Analysis- as required by CE Mark IVD Directive 98/79/EC - for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com.

12.2 Interpretation

1. ***Measurements and interpretation of results must be performed by a skilled individual or trained professional.***
2. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.
3. 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. Total serum triiodothyronine concentration is dependent upon a multiplicity of factors: thyroid gland function and its regulation, thyroxine binding globulin (TBG) concentration, and the binding of triiodothyronine to TBG (3, 4). **Thus, total triiodothyronine concentration alone is not sufficient to assess clinical status.**
7. A decrease in total triiodothyronine values is found with protein-wasting diseases, certain liver diseases and administration of testosterone, diphenylhydantoin or salicylates. A table of interfering drugs and conditions, which affect total triiodothyronine values, has been compiled by the Journal of the American Association of Clinical Chemists[3].

13.0 EXPECTED RANGES OF VALUES

A study of euthyroid adult population was undertaken to determine expected values for the T3 AccuBind™ ELISA Test System. The mean (R) values standard deviations (σ) and expected ranges (±2 σ) are presented in Table 1. The total number of samples was 105.

TABLE I
Expected Values for the T3 ELISA Test System (in ng/ml)

Mean (X)	**1.184**
Standard Deviation (σ)	0.334
Expected Ranges (±2 σ)	0.52 – 1.85

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 T3 AccuBind™ ELISA test system were determined by analyses on three different levels of pool control sera. The number (N), mean value (X), standard deviation (σ) and coefficient of variation (C.V.) for each of these control sera are presented in Table 2 and Table 3.

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

Sample	N	X	σ	C.V.
Low	16	0.78	0.06	7.9%
Normal	16	1.92	0.10	5.4%
High	16	3.55	0.14	3.9 %

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

Sample	N	X	σ	C.V.
Low	10	0.76	0.07	8.9%
Normal	10	1.85	0.13	6.7%
High	10	3.43	0.16	4.5%

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

14.2 Sensitivity

The T3 AccuBind™ ELISA test system has a sensitivity of 0.04 ng/ml. The sensitivity was ascertained by determining the variability of the 0 ng/ml serum calibrator and using the 2σ (95% certainty) statistic to calculate the minimum dose.

14.3 Accuracy

The T3 AccuBind™ ELISA method was compared with a reference radioimmunoassay method. Biological specimens from hypothyroid, euthyroid and hyperthyroid populations were used (The values ranged from 0.15ng/ml – 8.0ng/ml). The total number of such specimens was 120. The least square regression equation (y= mx+b) and the correlation coefficient were computed for the T3 AccuBind™ 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	1.62	y = 3.8 + 0.947(x)	0.987
Reference	1.68		

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 triiodothyronine 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 triiodothyronine needed to displace the same amount of conjugate.

Substance	Cross Reactivity	Concentration
l-Triiodothyronine	1.0000	-
l-Thyroxine	< 0.0002	10µg/ml
Iodothyrosine	< 0.0001	10µg/ml
Diiodothyrosine	< 0.0001	10µg/ml
Diiodothyronine	< 0.0001	10µg/ml
Phenylbutazone	< 0.0001	10µg/ml
Sodium Salicylate	< 0.0001	10µg/ml

15.0 REFERENCES

1. Gharib H., Ryan R.J, Mayberry W.E, & Hockett T., "Radioimmunoassay for Triiodothyronine (T3): Affinity and Specificity of Antibody for T3", *J Clinical Endocrinol.* **33,**509 (1971).
2. Chopra I.J., Ho R.S.,& Lam R. "An improved radio-immunoassay of triiodothyronine in T3 Calibrators", *J. Lab Clinical Med* **80,** 729 (1971).
3. Young D.S., Pestaner L.C., and Gilberman U., "Effects of Drugs on Clinical Laboratory Tests", *Clinical Chemistry* **21,** 3660 (1975).
4. Sterling L., "Diagnosis and Treatment of Thyroid Disease", *Cleveland CRC Press*, **p. 9-51** (1975).
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7. Comeau L., Pianan U., Leo-Mensah T, et.al.:"An automated chemiluminescent immunoassay test for total triiodothyronine", *Clin.Chem*.**37,** 941 (1991).
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9. Kozwich D., Davis G., Sockol C.:"Development of total triiodothyronine enzyme immunoassay in microtiter plate format", *Clin.Chem***. 37,** 1040 (1991).
10. Papanastasiou-Diamandi A., Khosravi M.:"Total T3 (triiodothyronine) measurement in serum by time resolved fluorescence immunoassay", *Clin.Chem***. 37,** 1029 (1991).

Revision: 4 **2022-May-01** **DCO: 1557**
Cat #: *125-300*

Size		96(A)	192(B)	480(D)	960(E)
Reagent (fill)	A)	1ml set	1ml set	2ml set	2ml set x2
	B)	1 (1.5ml)	2 (1.5ml)	1 (8ml)	2 (8ml)
	C)	1 (13ml)	2 (13ml)	1(60ml)	2 (60ml)
	D)	1 plate	2 plates	5 plates	10 plates
	E)	1 (20ml)	1 (20ml)	1 (60ml)	2 (60ml)
	F)	1 (7ml)	2 (7ml)	1 (30ml)	2 (30ml)
	G)	1 (7ml)	2 (7ml)	1 (30ml)	2 (30ml)
	H)	1 (8ml)	2 (8ml)	1 (30ml)	2 (30ml)

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Monobind Inc.
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AccuBind
ELISA Microwells

Total Thyroxine (T4) Test System
Product Code: 225-300

1.0 INTRODUCTION

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

2.0 SUMMARY AND EXPLANATION OF THE TEST

Measurement of serum thyroxine concentration is generally regarded as an important *in-vitro* diagnostic test for assessing thyroid function. This importance has provided the impetus for the significant improvement in assay methodology that has occurred in the last three decades. This procedural evolution can be traced from the empirical protein bound iodine (PBI) test (1) to the theoretically sophisticated radioimmunoassay (2).

This microplate enzyme immunoassay methodology provides the technician with optimum sensitivity while requiring few technical manipulations. In this method, serum reference, patient specimen, or control is first added to a microplate well. Enzyme-T4 conjugate is added, and then the reactants are mixed. A competition reaction results between the enzyme conjugate and the native thyroxine for a limited number of antibody combining sites immobilized on the well.

After the completion of the required incubation period, the antibody bound enzyme-thyroxine conjugate is separated from the unbound enzyme-thyroxine 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 thyroxine 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 thyroxine concentration.

3.0 PRINCIPLE

Competitive Enzyme Immunoassay (TYPE 5)
The essential reagents required for a solid phase enzyme immunoassay include immobilized antibody, enzyme-antigen conjugate and native antigen.

Upon mixing immobilized 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 insolubulized binding sites. The interaction is illustrated by the equation in the following below.

$$Enz_{Ag} + Ag + Ab_{C.W.} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} AgAb_{C.W.} + Enz_{AgAb_{C.W.}}$$

$Ab_{C.W}$ = Monospecific Immobilized Antibody (Constant Quantity)
Ag = Native Antigen (Variable Quantity)
Enz_{Ag} = Enzyme-antigen Conjugate (Constant Quantity)
$AgAb_{C.W.}$ = Antigen-Antibody Complex
$Enz_{Ag\,Ab_{C.W.}}$ = Enzyme-antigen Conjugate -Antibody Complex
k_a = Rate Constant of Association
k_{-a} = Rate Constant of Disassociation
$K = k_a / k_{-a}$ = Equilibrium Constant

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 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

A. T4 **Calibrators – 1ml/vial - Icons A-F**
Six (6) vials of serum reference for thyroxine at concentrations of 0 (**A**), 2.0 (**B**), 5.0 (**C**), 10.0 (**D**), 15.0 (**E**) and 25.0 (**F**) µg/dl. Store at 2-8°C. A preservative has been added. **For SI units: µg/dl x 12.9 = nmol/L**

B. T4-Enzyme Reagent – 1.5ml/vial - Icon Ⓔ
One (1) vial of thyroxine-horseradish peroxidase (HRP) conjugate in a bovine albumin-stabilizing matrix. A preservative has been added. Store at 2-8°C.

C. T3/T4 Conjugate Buffer – 13 ml - Icon Ⓑ
One (1) bottle reagent containing buffer, red dye, preservative, and binding protein inhibitors. Store at 2-8°C.

D. T4 Antibody Coated Plate – 96 wells - Icon
One 96-well microplate coated with sheep anti-thyroxine serum and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

E. Wash Solution Concentrate – 20ml - 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) bottle containing tetramethylbenzidine (TMB) in buffer. Store at 2-8°C.

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

H. Stop Solution – 8ml/vial - Icon STOP
One (1) bottle containing a strong acid (1.0N HCl). 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 2-8ºC. Kit and component stability are identified on the label.**
Note 3: Above reagents are for a 96-well microplate. For other kit configurations, see table at the end of this IFU.

4.1 Required But Not Provided:
1. Pipette capable of delivering 25µl & 50µl volumes with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100ml and 0.350ml volumes with a precision of better than 1.5%.
3. Adjustable volume (20-200µl) and (200-1000µl) dispenser(s) for conjugate and substrate preparation
4. Microplate washer or a squeeze bottle (optional).
5. Microplate Reader with 450nm and 620nm wavelength absorbance capability.
6. Test tubes for preparation of enzyme conjugate.
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 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 PEPARATION

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

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 of the specimen is required.

7.0 QUALITY CONTROL

Each laboratory should assay controls at levels in the hypothyroid, euthyroid and hyperthyroid 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. **Working Reagent A = T4-Enzyme Conjugate Solution**
   Dilute the T4-enzyme conjugate 1:11 with Total T3/T4 conjugate buffer in a suitable container. For example, dilute 160µl of conjugate with 1.6ml of buffer for 16 wells (A slight excess of solution is made). This reagent should be used within twenty-four hours for maximum performance of the assay. Store at 2-8°C.
   General Formula:
   Amount of Buffer required = Number of wells * 0.1
   Quantity of T4 Enzyme necessary = # of wells * 0.01
   i.e. = 16 x 0.1 = 1.6ml for Total T3/T4 conjugate buffer
   16 x 0.01 = 0.16ml (160µl) for T4 enzyme conjugate
2. **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.
3. **Working Substrate Solution**
   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 references and controls to room temperature (20 - 27°C).
Test Procedure should be performed by a skilled individual or trained professional*

1. Format the microplate's 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 2-8ºC.**
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 Working Reagent A, T4 Enzyme Reagent to all wells (see Reagent Preparation Section).
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. 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.**

Note: For reassaying specimens with concentrations greater than 25 µg/dl, pipet 12.5µl of the specimen and 12.5µl of the 0 serum reference into the sample well (this maintains a uniform protein concentration). Multiply the readout value by 2 to obtain the thyroxine concentration.

10.0 CALCULATION OF RESULTS

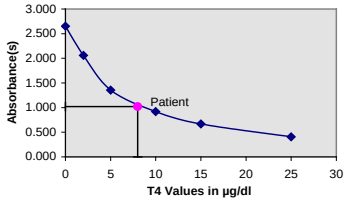
A dose response curve is used to ascertain the concentration of thyroxine in unknown specimens.
1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the absorbance for each duplicate serum reference versus the corresponding T4 concentration in µg/dl on linear graph paper (do not average the duplicates of the serum references before plotting).
3. Connect the points with a best-fit curve.
4. To determine the concentration of T4 for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in µg/dl) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance (1.022) intersects the standard curve at (8 µg/dl) T4 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.

Sample I.D.	Well Number	Abs (A)	Mean Abs (B)	Value (µg/dl)
Cal A	A1	2.648	2.650	0
	B1	2.652		
Cal B	C1	2.090	2.060	2
	D1	2.031		
Cal C	E1	1.344	1.355	5
	F1	1.366		
Cal D	G1	0.897	0.918	10
	H1	0.939		
Cal E	A2	0.676	0.668	15
	B2	0.659		
Cal F	C2	0.408	0.406	25
	D2	0.404		
Ctrl 1	E2	1.425	1.435	**4.6**
	F2	1.383		
Ctrl 2	G2	0.611	0.613	**16.3**
	H2	0.608		
Patient	A3	0.984	1.022	**8.0**
	B3	1.060		

EXAMPLE 1

Figure 1



The data presented in Example 1 and Figure 1 are 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 0 µg/dl should be ≥ 1.3.
2. Four out of six quality control pools should be within the established ranges.

12.0 RISK ANALYSIS

The MSDS and Risk Analysis Form for this product 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. Patient specimens with T4 concentrations greater than 35 µg/dl may be diluted ½ with the '0' serum reference into the sample well; pipet 12.5µl of the specimen and 12.5µl of the '0' serum reference in the sample well to maintain a uniform protein concentration. The sample's concentration is obtained by multiplying the result by the dilution factor, 2.
10. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from Monobind's IFU may yield inaccurate results.
11. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.
12. It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.
13. Risk Analysis- as required by CE Mark IVD Directive 98/79/EC - for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com.

12.2 Interpretation

1. ***Measurements and interpretation of results must be performed by a skilled individual or trained professional.***
2. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.
3. 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.
2. Total serum thyroxine concentration is dependent upon a multiplicity of factors: thyroid gland function and its regulation, thyroxine binding globulin (TBG) concentration, and the binding of thyroxine to TBG (3, 4). **Thus, total thyroxine concentration alone is not sufficient to assess clinical status.**
3. Total serum thyroxine values may be elevated under conditions such as pregnancy or administration of oral contraceptives. A T3 uptake test may be performed to estimate the relative TBG concentration in order to determine if the elevated T4 is caused by TBG variation.
4. A decrease in total thyroxine values is found with protein-wasting diseases, certain liver diseases and administration of testosterone, diphenylhydantoin or salicylates. A table of interfering drugs and conditions, which affect total thyroxine values, has been compiled by the Journal of the American Association of Clinical Chemists.

"NOT INTENDED FOR NEWBORN SCREENING"

13.0 EXPECTED RANGES OF VALUES

A study of euthyroid adult population was undertaken to determine expected values for the T4 AccuBind™ ELISA Test System. The mean (X) values, standard deviations (**σ**) and expected ranges (±2 **σ**) are presented in Table 1.

TABLE 1

Expected Values for the T4 ELISA Test System (in µg/dl)

	Male	Female *
Number of Specimens	42	58
Mean (X)	7.6	8.2
Std.Dev (σ)	1.6	1.7
Expected Ranges (±2 σ)	4.4 – 10.8	4.8 – 11.6

*Normal patients with high TBG levels were **not** excluded except if pregnant.

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 T4 AccuBind™ ELISA test system were determined by analyses on three different levels of pool control sera. The number (N), mean values (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 µg/dl)

Sample	N	X	σ	C.V.%
Low	20	6.87	0.16	2.3
Normal	20	9.95	0.16	1.6
High	20	13.13	0.17	1.3

TABLE 3

Between Assay Precision (Values in µg/dl)

Sample	N	X	σ	C.V.%
Low	20	5.76	0.37	6.3
Normal	20	9.41	0.57	6.1
High	20	16.18	1.21	7.5

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

14.2 Sensitivity

The T4 AccuBind™ ELISA test system has a sensitivity of 3.2ng/well. This is equivalent to a sample containing a concentration of 0.128 µg/dl. The sensitivity was ascertained by determining the variability of the 0 µg/dl serum calibrator and using the 2σ (95% certainty) statistic to calculate the minimum dose.

14.3 Accuracy

The tT4 AccuBind™ ELISA method was compared with a coated tube radioimmunoassay method. Biological specimens from hypothyroid, euthyroid and hyperthyroid populations were used (The values ranged from 0.8µg/dl – 25µg/dl). The total number of such specimens was 131. The least square regression equation and the correlation coefficient were computed for the T4 AccuBind™ 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	8.07	y = 0.39+0.952(x)	0.934
Reference	8.06		

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 thyroxine 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 thyroxine needed to displace the same amount of conjugate.

Substance	Cross Reactivity	Concentration
l–Thyroxine	1.0000	-
d-Thyroxine	0.9800	10µg/dl
d-Triiodothyronine	0.0150	100µg/dl
l–Triiodothyronine	0.0300	100µg/dl
Iodothyrosine	0.0001	100µg/ml
Diiodothyrosine	0.0001	100µg/ml
Diiodothyronine	0.0001	100µg/ml

15.0 REFERENCES

1. Barker S.B., "Determination of Protein Bound Iodine", *Journal Biological Chemistry* **173, 175** (1948).
2. Chopra I.J., Solomon D.H., Ho R.S., "A Radioimmunoassay of Thyroxine", J. Clinical EndocrinoL **33,** 865 (1971).
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5. Rae P, Farrar J, Beckett G, Toft A, "Assessment of thyroid status in elderly people". *British Med. Jour.* **307,**177-180.(1993).
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Revision: 4 **Date: 2022-May-01** **DCO: 1557**

Cat #: *225-300*

Size		96(A)	192(B)	480(D)	960(E)
Reagent (fill)	A)	1ml set	1ml set	2ml set	2ml set x2
	B)	1 (1.5ml)	2 (1.5ml)	1 (8ml)	2 (8ml)
	C)	1 (13ml)	2 (13ml)	1(60ml)	2 (60ml)
	D)	1 plate	2 plates	5 plates	10 plates
	E)	1 (20ml)	1 (20ml)	1 (60ml)	2 (60ml)
	F)	1 (7ml)	2 (7ml)	1 (30ml)	2 (30ml)
	G)	1 (7ml)	2 (7ml)	1 (30ml)	2 (30ml)
	H)	1 (8ml)	2 (8ml)	1 (30ml)	2 (30ml)

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

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Monobind Inc.
Lake Forest, CA 92630, USA

AccuBind
ELISA Microwells

Free Thyroxine (Free T4) Test System

Product Code: 1225-300

1.0 INTRODUCTION

Intended Use: The Quantitative Determination of Free Thyroxine Concentration in Human Serum by a Microplate Enzyme Immunoassay

2.0 SUMMARY AND EXPLANATION OF THE TEST

Thyroxine, the principal thyroid hormone, circulates in blood almost completely bound to carrier proteins. The main carrier is thyroxine-binding globulin (TBG). However, only the free (unbound) portion of thyroxine is responsible for the biological action. Further, the concentrations of the carrier proteins are altered in many clinical conditions, such as pregnancy. In normal thyroid function as the concentrations of the carrier proteins alters, the total thyroxine level changes so that the free thyroxine concentration remains constant. Thus, measurements of free thyroxine concentrations correlate better with clinical status than total thyroxine levels.

The increase in total thyroxine associated with pregnancy, oral contraceptives and estrogen therapy occasionally result in total T4 levels over the limits of normal while the free thyroxine concentration remains in the normal reference range. Masking of abnormal thyroid function can also occur in both hyper and hypothyroid conditions by alterations in the TBG concentration. The total T4 can be elevated or lowered by TBG changes such that the normal reference levels result. The free thyroxine concentration can help in uncovering the patient's actual clinical status.

In this method, serum reference, patient specimen, or control is first added to a microplate well. Enzyme-T4 conjugate (analog method) is added and the reactants are mixed. A competition reaction results between the enzyme conjugate and the free thyroxine for a limited number of antibody combining sites immobilized on the well.

After the completion of the required incubation period, the antibody bound enzyme-thyroxine conjugate is separated from the unbound enzyme-thyroxine conjugate via a wash step. 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 free thyroxine 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 free thyroxine concentration.

3.0 PRINCIPLE

Competitive Enzyme Immunoassay, Analog Method for Free-T4 (TYPE 5):

The essential reagents required for a solid phase enzyme immunoassay include immobilized antibody, enzyme-antigen conjugate and native antigen. Upon mixing immobilized antibody, enzyme-antigen conjugate and a serum containing the native free antigen, a competition reaction results between the native free antigen and the enzyme-antigen conjugate for a limited number of insolubilized binding sites. The interaction is illustrated by the followed equation:

$$Enz_{Ag} + Ag + Ab_{C.W.} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} AgAb_{C.W.} + {}^{Enz}AgAb_{C.W.}$$

$Ab_{C.W.}$ = Monospecific Immobilized Antibody (Constant Quantity)
Ag = Native Antigen (Variable Quantity)
^{Enz}Ag = Enzyme-antigen Conjugate (Constant Quantity)
$AgAb_{C.W.}$ = Antigen-Antibody Complex
$^{Enz}Ag\ Ab_{C.W.}$ = Enzyme-antigen Conjugate -Antibody Complex
K_a = Rate Constant of Association
k_{-a} = Rate Constant of Disassociation
$K = k_a / k_{-a}$ = Equilibrium Constant

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 inversely proportional to the native free antigen concentration. By utilizing several different serum references of known antigen con-centration, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

4.0 REAGENTS

Materials Provided:

A. Free T4 Calibrators – 1 ml/vial - Icons A-F
Six (6) vials of human serum based reference calibrators for free thyroxine at **approximate*** concentrations of 0 (**A**), 0.40 (**B**), 1.25 (**C**), 2.10 (**D**), 5.00 (**E**) and 7.40 (**F**) ng/dl. Store at 2-8°C. A preservative has been added. For SI units use the conversion factor 12.9 to convert ng/dl to pmol/L.
* Exact levels are given on the labels on a lot specific basis.

B. fT4- Enzyme Reagent – 13 ml/vial - Icon E
One (1) vial of thyroxine-horseradish peroxidase (HRP) conjugate in a protein-stabilized matrix. A preservative has been added. Store at 2-8°C.

C. Free T4 Antibody Coated Plate – 96 wells - Icon
One 96-well microplate coated with anti-thyroxine serum and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

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

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

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

G. Stop Solution – 8 ml/vial - Icon STOP
One (1) bottle 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: Opened reagents are stable for sixty (60) days when stored at 2-8°C. **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 96-well microplate. For other kit configurations, please refer to the table at the end of this IFU.

4.1 Materials Required But Not Provided:

1. Pipette capable of delivering 50µl & 100µl volumes with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100ml and 0.350ml 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.

Samples may be refrigerated at 2-8^{0}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^{0}C for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.100ml of the specimen is required.

7.0 QUALITY CONTROL

Each laboratory should assay controls at levels in the hypothyroid, euthyroid and hyperthyroid 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. Diluted buffer can be stored at 2-30°C for up to 60 days.
2. **Working Substrate Solution**
   Pour the contents of the plastic 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 references 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, 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, control or specimen into the assigned well.
3. Add 0.100 ml (100µl) of fT4 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 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.**

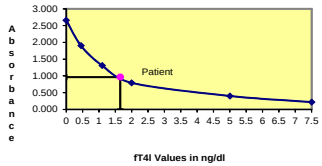
10.0 CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of free T4 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 Free T4 concentration in ng/dl on linear graph paper (do not average the duplicates of the serum references before plotting).
3. Connect the points with a best-fit curve.
4. To determine the concentration of Free T4 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/dl) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance (0.964) intersects the dose response curve at (1.65ng/dl) free T4 concentration (See 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. **Assigned values for calibrators are lot specific.**

Figure 1



EXAMPLE 1

Sample I.D.	Well Number	Abs (A)	Mean Abs (B)	Value* (ng/dl)
Cal A	A1	2.658	2.612	0.00
	B1	2.566		
Cal B	C1	1.919	1.900	0.45
	D1	1.880		
Cal C	E1	1.339	1.306	1.10
	F1	1.273		

Cal D	G1	0.769	0.790	2.00
	H1	0.811		
Cal E	A2	0.396	0.400	5.00
	B2	0.404		
Cal F	C2	0.215	0.217	7.40
	D2	0.219		
Ctrl 1	E2	1.827	1.835	**0.50**
	F2	1.843		
Ctrl 2	G2	0.541	0.557	**2.70**
	H2	0.573		
Patient	A3	0.951	0.964	**1.65**
	B3	0.976		

Note 1: 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.

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 ng/dl should be ≥ 1.3.
2. Four out of six quality control pools should be within the established ranges.

12.0 RISK ANALYSIS

The MSDS and Risk Analysis Form for this product 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. 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. If a patient, for some reason, reads higher than the highest calibrator report as such (e.g. > 7.4 ng/dl). ***Do not try to dilute the sample. TBG variations in different matrices will not allow Free T4 hormone to dilute serially.***
7. Serum free-thyroxine concentration is dependent upon a multiplicity of factors: thyroid gland function and its regulation, Thyroxine binding globulin (TBG) concentration, and the binding of Thyroxine to TBG (3, 4). Thus, free-Thyroxine concentration alone is not sufficient to assess the clinical status.
8. Serum free-thyroxine values may be elevated under conditions such as pregnancy or administration of oral contraceptives.
9. A decrease in free thyroxine values is found with protein-wasting diseases, certain liver diseases and administration of testosterone, diphenylhydantoin or salicylates. A table of interfering drugs and conditions, which affect free Thyroxine values, has been compiled by the Journal of the American Association of Clinical Chemists.
10. The interpretation of Free T4 is complicated by a variety of drugs that can affect the binding of T4 to the thyroid hormone carrier proteins or interfere in its metabolism to T3. In severe non-thyroidal illness (NTI) the assessment of thyroid becomes especially difficult. Since the patients in this category may suffer from concomitant primary hypothyroidism or from compensatory secondary hypothyroidism. In cases like these a sensitive TSH evaluation of the patient may be recommended. Please see Monobind Cat# 325-300.
11. In rare conditions associated with extreme variations in albumin binding capacity for T4- such as familial dysalbuminemic hyperthyroxinemia (FDH) – direct assessment of Free T4 may be misleading.
12. Circulating antibodies to T4 and hormone binding inhibitors may interfere in the performance of the assay.
13. Heparin is reported to have in vivo and in vitro effects on free T4 levels. Samples from patients undergoing heparin therapy should be collected well before the administration of the anticoagulant.

"NOT INTENDED FOR NEWBORN SCREENING"

13.0 EXPECTED RANGES OF VALUES

A study of euthyroid adult population was undertaken to determine expected values for the Free T4 AccuBind® ELISA test system. The mean (X) values, standard deviations (σ) and expected ranges (±2σ) are presented in Table 1.

TABLE 1
Expected Values for Free T4 ELISA Test System (in ng/dl)

	Adult	Pregnancy
Number of Specimens	89	31
Mean (X)	1.40	1.50
Standard Deviation (σ)	0.30	0.37
Expected Ranges (±2 σ)	0.8 – 2.0	0.76 – 2.24

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 *inter* and *intra* assay precisions of the Free T4 AccuBind® ELISA test system were determined by analyses on three different levels of pooled patient sera. The number (n), mean values (x), standard deviation (σ) and coefficient of variation (C.V.) for each of these control sera are presented in Table 2 and Table 3.

In order to validate the *intra-assay* precision of the Free T4 AccuBind® ELISA test system, twenty replicates of each of three pooled sera (low medium and high ranges of the dose response curve) were assayed in the same assay. An intra-assay precision of 3.25 to 10.98% was obtained.

TABLE 2
Intra-Assay Precision (in ng/dl)

Sample	N	X	σ	C.V.
Low	20	0.550	0.061	10.98%
Medium	20	1.740	0.074	4.26%
High	20	3.250	0.106	3.25%

In order to validate the *inter-assay* precision of fT4 AccuBind® ELISA test system, one duplicate of each of three pooled sera (low medium and high ranges of the dose response curve) was assayed in 10 assays done over a period of six months that involved five different sets of reagents and three different technicians. An inter-assay precision of 6.01 to 10.81% was obtained.

TABLE 3
Inter-Assay Precision (in ng/dl)

Sample	N	X	σ	C.V.
Low	10	0.480	0.052	10.81%
Medium	10	1.410	0.085	6.01%
High	10	3.490	0.279	7.90%

14.2 Sensitivity

The Free T4 AccuBind® ELISA test system has a sensitivity of 0.162 ng/dl. The sensitivity was ascertained by determining the variability of the 0 ng/dl serum calibrator and using the 2σ (95% certainty) statistics to calculate the minimum dose.

14.3 Accuracy

The Free T4 AccuBind® ELISA test system was compared with a coated tube radioimmunoassay (**RIA)** method. Biological specimens from hypothyroid, euthyroid and hyperthyroid populations were used (The values ranged from 0.1ng/dl – 8ng/dl). The total number of such specimens was 197. The least square regression equation and the correlation coefficient were computed for this Free T4 AccuBind® ELISA method in comparison with the predicate method (Table 4).

TABLE 4
Linear Regression Analysis

Method	Mean (x)	Equation	Correlation Coefficient
Monobind EIA "X"	1.56	y = 0.1034 + 0.9525x	0.920
Predicate RIA "Y"	1.59		

Only slight amounts of bias between this method and the reference method are indicated by the closeness of the mean values.

14.4 Specificity:

The cross-reactivity of the thyroxine antibody used for Free T4 AccuBind® ELISA to selected substances was evaluated by adding massive amounts of the interfering substance to a serum matrix. The cross-reactivity was calculated by deriving a ratio between doses of interfering substance to dose of thyroxine needed to displace the same amount of the conjugate.

Substance	Cross Reactivity	Concentration
l–Thyroxine	1.0000	----
d-Thyroxine	0.9800	10µg/dl
d-Triiodothyronine	0.0150	100µg/dl
l–Triiodothyronine	0.0300	100µg/dl
Iodothyrosine	0.0001	100µg/ml
Diiodotyrosine	0.0001	100µg/ml
Diiodothyronine	0.0001	100µg/ml
TBG	N/D	40 µg/ml
Albumin	N/D	40 mg/ml
Phenylbutazone	N/D	10 µg/ml
Phenytoin	N/D	40 µg/ml
Salicylates	N/D	500 µg/ml

15.0 REFERENCES

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11. Wosilait WD, "A theoretical analysis of the distribution of thyroxine among sites on the thyroxine binding globulin, thyroid binding prealbumin and serum albumin", *Res Comm Chem Pathology-Pharmacology,* **16,** 541-548 (1977).

Revision: 6 **Date: 2022-MAY-01** **DCO: 1557**
Cat #: *1225-300*

Size		96(A)	192(B)	480(D)	960(E)
Reagent (fill)	A)	1ml set	1ml set	2ml set	2ml set x2
	B)	1 (13ml)	2 (13ml)	1(60ml)	2 (60ml)
	C)	1 plate	2 plates	5 plates	10 plates
	D)	1 (20ml)	1 (20ml)	1 (60ml)	2 (60ml)
	E)	1 (7ml)	2 (7ml)	1 (30ml)	2 (30ml)
	F)	1 (7ml)	2 (7ml)	1 (30ml)	2 (30ml)
	G)	1 (8ml)	2 (8ml)	1 (30ml)	2 (30ml)

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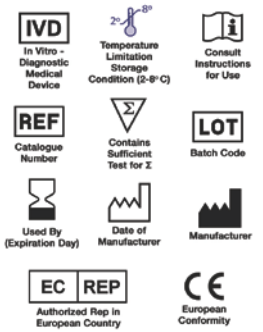
Please visit our website to learn more about our products and services.

Glossary of Symbols
(EN 980/ISO 15223)



Monobind Inc.
Lake Forest, CA 92630, USA

AccuBind
ELISA Microwells

Free Triiodothyronine (Free T3) Test System

Product Code: 1325-300

1.0 INTRODUCTION

Intended Use: The Quantitative Determination of Free Triiodothyronine Concentration in Human Serum by a Microplate Enzyme Immunoassay. Levels of Free T3 are thought to reflect the amount of T3 available to the cells and may therefore determine the clinical metabolic status of T3.

2.0 SUMMARY AND EXPLANATION OF THE TEST

Triiodothyronine, a thyroid hormone, circulates in blood bound to carrier proteins (1,2). The main transport protein is thyroxine-binding globulin (TBG). However, only the free (unbound) portion of triiodothyronine is believed to be responsible for the biological action. Further, the concentrations of the carrier proteins are altered in many clinical conditions, such as pregnancy. In normal thyroid function as the concentrations of the carrier proteins alters, the total triiodothyronine level changes so that the free triiodothyronine concentration remains constant. Thus, measurements of free triiodothyronine concentrations correlate more reliably with clinical status than total triiodothyronine levels.

For example, the increase in total triiodothyronine levels associated with pregnancy, oral contraceptives and estrogen therapy result in higher total T3 levels while the free T3 concentration remains basically unchanged.

This microplate enzyme immunoassay methodology provides the technician with optimum sensitivity while requiring few technical manipulations in a direct determination of free T3. In this method, serum reference, patient specimen, or control is first added to a microplate well. Enzyme-T3 conjugate (analog method) is added, and then the reactants are mixed. A competition reaction results between the enzyme conjugate and the free triiodothyronine for a limited number of antibody combining sites immobilized on the well.

After the completion of the required incubation period, the antibody bound enzyme-triiodothyronine conjugate is separated from the unbound enzyme-triiodothyronine 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 free triiodothyronine 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 free triiodothyronine concentration.

3.0 PRINCIPLE

Competitive Enzyme Immunoassay TYPE 5
(Analog Method for Free T3)
The essential reagents required for a solid phase enzyme immunoassay include immobilized T3 antibody, enzyme-T3 conjugate and native free T3 antigen. The enzyme-T3 conjugate should have no measurable binding to serum proteins especially TBG and albumin. The method achieves this goal.

Upon mixing immobilized antibody, enzyme-T3 conjugate and a serum containing the native free T3 antigen, a competition reaction results between the native free T3 and the enzyme-T3 conjugate for a limited number of insolubulized binding sites. The interaction is illustrated by the following equation:

$$Enz_{Ag} + Ag + Ab_{C.W.} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} AgAb_{C.W.} + {}^{Enz}AgAb_{C.W.}$$

$Ab_{C.W.}$ = Monospecific Immobilized Antibody (Constant Quantity)

Ag = Native Antigen (Variable Quantity)

Enz$_{Ag}$ = Enzyme-antigen Conjugate (Constant Quantity)

$AgAb_{C.W.}$ = Antigen-Antibody Complex

Enz$_{Ag}$ $Ab_{C.W.}$ = Enzyme-antigen Conjugate -Antibody Complex

k_a = Rate Constant of Association

k_{-a} = Rate Constant of Disassociation

$K = k_a / k_{-a}$ = Equilibrium Constant

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 inversely proportional to the native free antigen concentration. By utilizing several different serum references of known antigen concentration, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

4.0 REAGENTS

Materials Provided

A. Free T3 Calibrators – 1ml/vial - Icons A-F
Six (6) vials of serum reference for free triiodothyronine at **approximate*** concentrations of 0 (**A**), 1.0 (**B**), 3.0 (**C**), 5.0 (**D**), 8.0 (**E**) and 16.0 (**F**) pg/ml. Store at 2-8°C. A preservative has been added. For SI units use the conversion factor 1.536 to convert pg/ml to pmol/L.
* Exact levels are given on the labels on a lot specific basis.

B. Free T3- Enzyme Reagent – 13ml/vial - Icon
One (1) vial of triiodothyronine -horseradish peroxidase (HRP) conjugate in a bovine albumin-stabilizing matrix. A preservative has been added. Store at 2-8°C.

C. T3 Antibody Coated Plate – 96 wells - Icon
One 96-well microplate coated with sheep anti-triiodothyronine serum and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

D. Wash Solution Concentrate – 20ml - 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) bottle containing tetramethylbenzidine (TMB) in buffer. Store at 2-8°C.

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

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

H. Product Instructions.

4.1 Required But Not Provided:
1. Pipette capable of delivering 50µl volumes with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100ml and 0.350ml 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.

Note 1: Do not use reagents beyond the kit expiration date.
Note 2: Opened reagents are stable for sixty (60) days when stored at 2-8°C. **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 96-well microplate. For other kit configurations, please see table at end of this IFU.

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 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.

Samples may be refrigerated at 2-8^{0}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^{0}C 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 hypothyroid, euthyroid and hyperthyroid 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 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**
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 references 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, 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, control or specimen into the assigned well.
3. Add 0.100 ml (100µl) of fT3-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 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 free triiodothyronine 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 Free T3 concentration in pg/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 Free T3 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 (1.855) (intersects the standard curve at (2.1pg/ml) Free T3 concentration (See Figure 1).

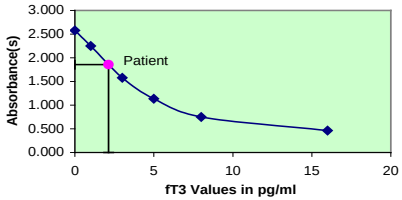
Note: Computer data reduction software designed for ELISA assays may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained.

EXAMPLE 1

Sample I.D.	Well Number	Abs (A)	Mean Abs (B)	Value* (pg/ml)
Cal A	A1	2.658	2.579	0.0
	B1	2.531		
Cal B	C1	2.264	2.248	1.0
	D1	2,233		
Cal C	E1	1.570	1.578	3.0
	F1	1.585		
Cal D	G1	1.124	1.135	5.0
	H1	1.145		
Cal E	A2	0.749	0.748	8.0
	B2	0.748		
Cal F	C2	0.463	0.463	16.0
	D2	0.462		
Patient	E2	1.860	1.855	***2.1***
	F2	1.849		

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**. Assigned values for calibrators are lot specific.**

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 A 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. 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. If a patient, for some reason, reads higher than the highest calibrator report as such (e.g. > 16pg/ml). ***Do not try to dilute the sample. TBG variations in different matrices will not allow Free T3 hormone to dilute serially.***
7. Several drugs are known to affect the binding of Triiodothyronine to the thyroid hormone carrier proteins or its metabolism to T3 and complicate the interpretation of free T3 results (3).
8. Circulating autoantibodies to T3 and hormone-binding inhibitors may interfere (4).
9. Heparin has been reported to have *in vivo* and *in vitro* effects on free T3 concentration (5). Therefore, do not obtain samples in which this anti-coagulant has been used.
10. In severe nonthyroidal illness (NTI), the assessment of thyroid status becomes very difficult. TSH measurements are recommended to identify thyroid dysfunction (6).
11. Familial dysalbuminemic conditions may yield erroneous results on direct free T3 assays (7).

"NOT INTENDED FOR NEWBORN SCREENING"

13.0 EXPECTED RANGES OF VALUES

A study of euthyroid adult population was undertaken to determine expected values for the fT3 AccuBind™ ELISA test system. The mean values (X), standard deviations (σ.) and expected ranges (±2σ.) are presented in Table 1.

TABLE I
Expected Values for Free T3 ELISA Test System (in pg/ml)

	Adult	Pregnancy
Number Specimens	110	75
Mean (X)	2.8	3.0
Standard Deviation (σ)	0.7	0.6
Expected Ranges (±2 σ)	1.4 – 4.2	1.8 – 4.2

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 Free T3 AccuBind™ ELISA test system were determined by analyses on three different levels of pool control sera. The number (N), mean values (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 pg/ml)

Sample	N	X	σ	C.V.
Low	24	1.85	0.09	4.9%
Normal	24	4.49	0.16	3.6%
High	24	8.00	0.25	3.1%

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

Sample	N	X	σ	C.V.
Low	12	2.16	0.29	13.1%
Normal	12	5.09	0.40	7.9%
High	12	9.13	0.94	10.2%

*As measured in twelve experiments in duplicate.

14.2 Sensitivity

The FreeT3 AccuBind™ ELISA test system has a sensitivity of 0.410 pg/ml. The sensitivity was ascertained by determining the variability of the 0 pg/ml serum calibrator and using the 2 σ (95% certainty) statistic to calculate the minimum dose.

14.3 Accuracy

The Free T3 AccuBind™ ELISA test system was compared with a coated tube radioimmunoassay analog method. Biological specimens from hypothyroid, euthyroid and hyperthyroid populations were used (The values ranged from 0.1pg/ml – 14pg/ml). The total number of such specimens was 151. The least square regression equation and the correlation coefficient were computed for this Free T3 AccuBind™ 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 (Y)	3.05	y = 0.35+0.922(x)	0.902
Reference (X)	2.92		

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 triiodothyronine 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 triiodothyronine needed to displace the same amount of conjugate.

SUBSTANCE	Cross Reactivity	Concentration
L-Triiodothyronine	1.0000	----
L-Thyroxine	< 0.0002	10 µg/ml
Iodothyrosine	< 0.0001	10 µg/ml
Diiodothyrosine	< 0.0001	10 µg/ml
Diiodothyronine	< 0.0001	10 µg/ml
Phenylbutazone	< 0.0001	10 µg/ml
Sodium Salicylate	< 0.0001	10 µg/ml

15.0 REFERENCES

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Revision: 5 **Date: 2022-May-01** **DCO: 1557**

Cat #: *1325-300*

Size		96(A)	192(B)	480(D)	960(E)
Reagent (fill)	A)	1ml set	1ml set	2ml set	2ml set x2
	B)	1 (13ml)	2 (13ml)	1(60ml)	2 (60ml)
	C)	1 plate	2 plates	5 plates	10 plates
	D)	1 (20ml)	1 (20ml)	1 (60ml)	2 (60ml)
	E)	1 (7ml)	2 (7ml)	1 (30ml)	2 (30ml)
	F)	1 (7ml)	2 (7ml)	1 (30ml)	2 (30ml)
	G)	1 (8ml)	2 (8ml)	1 (30ml)	2 (30ml)

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IVD 2°-8° CE
EC REP **CEpartner4U,** Esdoornlaan 13
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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 2-8°C. Kit and component stability are identified on label.**
Note 3: Above reagents are for a single 96-well microplate.

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

5.0 PRECAUTIONS

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

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

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

6.0 SPECIMEN COLLECTION AND PREPARATION

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

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

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

7.0 QUALITY CONTROL

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

8.0 REAGENT PREPARATION

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

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

9.0 TEST PROCEDURE

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

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

10.0 CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of IgE in unknown specimens.
1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the absorbance for each duplicate serum reference versus the corresponding IgE concentration in IU/ml on linear graph

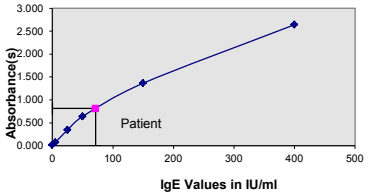
paper (do not average the duplicates of the serum references before plotting).
3. Draw the best-fit curve through the plotted points.
4. To determine the concentration of IgE for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in IU/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance (1.323) intersects the dose response curve at 142 IU/ml IgE concentration (See Figure 1).

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

EXAMPLE 1

Sample I.D.	Well	Abs	Mean Abs (B)	Conc
Cal A	A1	0.014	0.015	0
	B1	0.016		
Cal B	C1	0.072	0.073	5
	D1	0.074		
Cal C	E1	0.364	0.345	25
	F1	0.326		
Cal D	G1	0.663	0.639	50
	H1	0.614		
Cal E	A2	1.340	1.364	150
	B2	1.388		
Cal F	C2	2.601	2.641	400
	D2	2.682		
Ctrl 1	E2	2.575	2.562	***375.3***
	F2	2.549		
Ctrl 2	G2	0.818	0.813	***71.2***
	H2	0.807		
Patient 1	A3	1.322	1.323	***142.0***
	B3	1.324		

Figure 1



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

11.0 Q.C. PARAMETERS

In order for the assay results to be considered valid the following criteria should be met:
1. The absorbance (OD) of calibrator 'A' should be ≤ 0.05
2. The absorbance (OD) of calibrator 'F' should be ≥ 1.3
3. Four out of six quality control pools should be within the established ranges.

12.0 RISK ANALYSIS

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

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

12.2 Interpretation
1. ***Measurements and interpretation of results must be performed by a skilled individual or trained professional.***
2. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.
3. The reagents for the test system have been formulated to eliminate maximal interference; however, potential interaction between rare serum specimens and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for all kinds of immunoassays (Boscato LM, Stuart MC. 'Heterophilic antibodies: a problem for all immunoassays' Clin. Chem. 1988:3427-33). For diagnostic purposes, the results from this assay should be in combination with clinical examination, patient history and all other clinical findings.
4. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
5. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, Monobind shall have no liability.
6. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.
7. Serum IgE concentration is dependent upon a multiplicity of factors: including if the patient is sensitized, how many times the patient has been exposed to a specific allergen etc. Total IgE concentration alone is not sufficient to assess the clinical status. All the clinical findings especially specific allergy testing should be taken into consideration while determining the clinical status of the patient.
8. Since all atopic reactions are not IgE mediated, all relevant clinical information should be taken into consideration before making any determination for patients who may be in the normal range.

13.0 EXPECTED RANGES OF VALUES

A study of population from different age groups was conducted to evaluate the IgE AccuBind® ELISA test system. The results are presented in Table 1:

TABLE 1
Expected Values for the IgE (In IU/ml)

Age (Yrs)	Number (n)	Median	Absolute Range
0-3	31	6.4	ND - 46
3-16	43	25.0	ND – 280
Adult	145	43	0 - 200

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

14.0 PERFORMANCE CHARACTERISTICS

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

TABLE 2
Intra-Assay Precision (in IU/ml)

SAMPLE	N	X	σ	C.V.%
Low	20	48.9	2.87	5.87
Medium	20	160.5	6.47	4.03
High	20	297.6	5.81	1.95

TABLE 3
Inter Assay Precision (in IU/ml)

SAMPLE	N	X	σ	C.V.%
Low	10	46.3	3.9	8.42
Medium	10	157.0	7.3	4.64
High	10	301.0	10.6	3.52

14.2 Sensitivity
The IgE AccuBind® ELISA test system has a sensitivity of 0.125 IU/ml. The sensitivity was ascertained by determining the variability of the 0 IU/ml serum calibrator and using the 2σ (95% certainty) statistics to calculate the minimum dose.

14.3 Accuracy
The IgE AccuBind® ELISA test system was compared with a reference method. Biological specimens with IgE levels in the low, medium and high ranges were used. The values ranged from 0.8 to 3100 IIU/ml. The total number of such specimens was 219. The least square regression equation and the correlation coefficient were computed for this IgE AccuBind® ELISA method in comparison with the predicate method (Table 4):

TABLE 4

Method	Mean	Least Square Regression Analysis	Correlation Coefficient
Monobind (X)	179	x= -12.9 + 1.21(Y)	0.967
Predicate (Y)	157		

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

14.4 Specificity
The specificity of the IgE AccuBind® ELISA test system, to closely related immunoglobulins was evaluated by adding those at twice the physiological concentrations to a serum matrix. No cross-reaction between the antibodies used and the related molecules was detected.

14.5. High Dose Effect
Since the assay is sequential in design, high concentrations of IgE do not show the hook effect. Myeloma IgE patient samples with concentrations over 8 million IU/ml demonstrated extremely high levels of absorbance.

14.6 Linearity
Two patient pools were assayed diluted (in 'A' Calibrator) and undiluted with the IgE AccuBind® ELISA test system. The observed and expected values are listed below in Table 5:

TABLE 5

Sample	Observed (O) (IU/ml)	Expected (E) (IU/ml)	% Recovery (O/E)
Pool 1	106.8	-	-
Pool 1/2	50.8	53.4	95.1
Pool 1/4	25.3	26.7	94.8
Pool 1/8	13.4	13.3	100.6
Pool 1/16	6.6	6.7	98.5
Pool 2	395.9	-	-
Pool 2/2	189.5	197.9	95.8
Pool 2/4	106.1	98.9	107.2
Pool 2/8	48.0	49.5	96.9
Pool 2/16	25.8	24.7	104.2

14.7 Recovery
Two patient pools were spiked with known amounts of IgE and assayed with the IgE AccuBind® ELISA test system. The observed and expected values are listed below in Table 6.

TABLE 6

Sample	Observed (O) (IU/ml)	Expected (E) (IU/ml)	% Recovery (O/E)
Pool 1	25.7	-	-
Pool 1+ 25	50.7	50.7	100.0
Pool 1+ 50	74.8	75.7	101.2
Pool 1+ 100	122.7	125.7	97.6
Pool 1+ 200	232.0	225.7	102.7
Pool 2	12.3	-	-
Pool 2 + 25	41.7	37.3	111.2
Pool 2+ 50	62.6	62.3	100.6
Pool 2+ 100	109.4	112.3	97.4
Pool 2+ 200	197.2	212.3	92.8

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MP2525 Product Code: *2525-300*

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Glossary of Symbols
(EN 960/ISO 15223)

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

Monobind Inc.
Lake Forest, CA 92630, USA

AccuBind
ELISA Microwells

C-Peptide Test System
Product Code: 2725-300

1.0 INTRODUCTION

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

2.0 SUMMARY AND EXPLANATION OF THE TEST

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

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

3.0 PRINCIPLE

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

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

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

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

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

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

4.0 REAGENTS AND MATERIALS PROVIDED

Materials Provided:

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

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

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

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

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

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

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

H. Product Instructions.

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

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

5.0 PRECAUTIONS

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

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

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

6.0 SPECIMEN COLLECTION AND PREPARATION

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

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

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

7.0 QUALITY CONTROL

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

8.0 REAGENT PREPARATION

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

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

9.0 TEST PROCEDURE

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

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

DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION

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

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

10.0 CALCULATION OF RESULTS

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

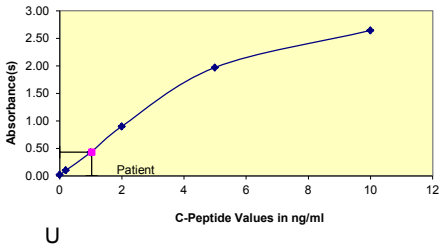
Note: Computer data reduction software designed for ELISA assays may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained.

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

EXAMPLE 1

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

Figure 1



U

11.0 QC PARAMETERS

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

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

12.0 RISK ANALYSIS

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

12.1 Assay Performance

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

12.2 Interpretation

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

13.0 EXPECTED VALUES

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

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

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

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

Adult (Normal)	**0.7 – 1.9 ng/ml**

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

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

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

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

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

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

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

14.2 Sensitivity

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

14.3 Accuracy

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

TABLE 4

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

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

14.4 Specificity

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

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

15.0 REFERENCES

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MP2725 Product Code: 2725-300

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

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

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

Glossary of Symbols
(EN 980/ISO 15223)

IVD — In Vitro - Diagnostic Medical Device

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

Consult Instructions for Use

REF — Catalogue Number

Σ — Contains Sufficient Test for Σ

LOT — Batch Code

Used By (Expiration Day)

Date of Manufacturer

Manufacturer

EC REP — Authorized Rep in European Country

CE — European Conformity

Monobind Inc.
Lake Forest, CA 92630, USA

AccuBind
ELISA Microwells

Cancer Antigen 125 (CA-125) Test System

Product Code: 3025-300

1.0 INTRODUCTION

Intended Use: The Quantitative Determination of Cancer Antigen 125 (CA-125) Concentration in Human Serum by a Microplate Enzyme Immunoassay, Colorimetric

2.0 SUMMARY AND EXPLANATION OF THE TEST

Cancer Antigen 125 (CA-125) is a glycoprotein that occurs in blood as high molecular weight entity (MB_{rB} > *200*,000). High concentrations of this antigen are associated with ovarian cancer and a range of benign and malignant diseases. Although the specificity and sensitivity of CA-125 assays are somewhat limited, especially in early diagnosis of ovarian cancer, the assay has found widespread use in the differential diagnosis of adnexal masses, in monitoring disease progression and response to therapy in ovarian cancer, and in the early detection of recurrence after surgery or chemotherapy for ovarian cancer. Published literature has shown that elevated serum CA-125 levels can be observed in patients with serious endometroid, clear cell and undifferentiated ovarian carcinoma. The serum CA-125 is elevated in 1% of normal healthy women, 3% of normal healthy women with benign ovarian diseases, and 6% of patients with non-neoplastic conditions (including, but not limited to, first trimester pregnancy, menstruation, endometriosis uterine fibrosis, acute salphingitis, hepatic diseases and inflammation of peritoneum or pericardium).

In this method, CA-125 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 CA-125) are added and the reactants mixed. Reaction between the various CA-125 antibodies and native CA-125 forms a sandwich complex that binds with the streptavidin coated to the well.

After the completion of the required incubation period, the enzyme-CA-125 antibody bound conjugate is separated from the unbound enzyme-CA-125 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 CA-125 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 CA-125 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-CA-125 antibody.

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

$$^{Enz}Ab + Ag_{CA\text{-}125} + {}^{Btn}Ab_{(m)} \underset{k\text{-}a}{\overset{k_a}{\rightleftharpoons}} {}^{Enz}Ab - Ag_{CA\text{-}125} - {}^{Btn}Ab_{(m)}$$

$^{Btn}Ab_{(m)}$ = Biotinylated Monoclonal Antibody (Excess Quantity)
$Ag_{CA\text{-}125}$ = Native Antigen (Variable Quantity)
^{Enz}Ab = Enzyme labeled Antibody (Excess Quantity)
$^{Enz}Ab - Ag_{CA\text{-}125} - {}^{Btn}Ab_{(m)}$ = Antigen-Antibodies Sandwich Complex
k_a = Rate Constant of Association
k_{-a} = Rate Constant of Dissociation

Simultaneously, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antibody. This interaction is illustrated below:
$^{Enz}Ab - Ag_{CA\text{-}125} - {}^{Btn}Ab_{(m)} + Streptavidin_{CW} \Rightarrow$ Immobilized complex
$Streptavidin_{CW}$ = Streptavidin immobilized on well
Immobilized complex = sandwich complex bound to the well

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

4.0 REAGENTS

Materials Provided:

A. CA-125 Calibrators - 1ml/vial- Icons A-F
Six (6) vials of references CA-125 Antigen at levels of 0(**A**), 15(**B**), 50(**C**), 100(**D**), 200(**E**) and 400(**F**) U/ml. A preservative has been added. Store at 2-8°C.

Note: The human serum based standards were made using a >99% pure affinity purified preparation of CA-125. The preparation was calibrated against Centocor CA-125 IRMA test.

B. CA-125 Enzyme-Reagent – 13ml/vial - Icon Ⓔ
One (1) vial containing enzyme labeled antibody, biotinylated monoclonal mouse IgG in buffer, dye, and preservative. Store at 2-8°C.

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

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

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

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

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 0.025 & 0.050ml (25 & 50µl) volumes with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100 & 0.350ml (100 & 350µl) volumes with a precision of better than 1.5%.
3. Microplate washers or a squeeze bottle (optional).
4. Microplate Reader with 450nm and 620nm wavelength absorbance capability.
5. Absorbent Paper for blotting the microplate wells.
6. Plastic wrap or microplate cover for incubation steps.
7. Vacuum aspirator (optional) for wash steps.
8. Timer.
9. Quality control materials

5.0 PRECAUTIONS

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

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

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

6.0 SPECIMEN COLLECTION AND PREPARATION

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

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

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

7.0 QUALITY CONTROL

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

8.0 REAGENT PREPARATION

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

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 0.025ml (25µl) of the appropriate serum reference calibrator, control or specimen into the assigned well.
3. Add 0.100ml (100µl) of the CA-125 Enzyme Reagent to each well. **It is very important to dispense all reagents close to the bottom of the coated well.**
4. Swirl the microplate gently for 20-30 seconds to mix and cover.
5. Incubate 60 minutes at room temperature.
6. Discard the contents of the microplate by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper.
7. Add 0.350ml (350µl) of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.**
8. Add 0.100ml (100µl) of working substrate solution to all wells (see Reagent Preparation Section). **Always add reagents in the same order to minimize reaction time differences between wells.**

DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION

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

10.0 CALCULATION OF RESULTS

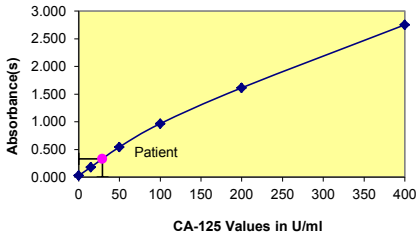
A dose response curve is used to ascertain the concentration of CA-125 in unknown specimens.
1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the absorbance for each duplicate serum reference versus the corresponding CA-125 concentration in U/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 CA-125 for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in U/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance (0.331l) intersects the dose response curve at 29.3U/ml CA-125 concentration (See Figure 1).

Note: Computer data reduction software designed 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 (U/ml)
Cal A	A1	0.035	0.029	0
	B1	0.022		
Cal B	C1	0.186	0.182	15
	D1	0.178		
Cal C	E1	0.536	0.545	50
	F1	0.554		
Cal D	G1	0.985	0.967	100
	H1	0.949		
Cal E	A2	1.615	1.615	200
	B2	1.616		
Cal F	C2	2.749	2.753	400
	D2	2.758		
Patient	A3	0.336	0.331	**29.3**
	B3	0.325		

Figure 1



*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.

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

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 CA-125 concentrations above 400 U/ml may be diluted (for example 1/10 or higher) with normal male serum (CA-125 < 5 U/ml) and re-assayed. The sample's concentration is obtained by multiplying the result by the dilution factor (10).
10. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from Monobind's IFU may yield inaccurate results.
11. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.
12. It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.
13. Risk Analysis - as required by CE Mark IVD Directive 98/79/EC - for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com.

12.2 Interpretation

1. 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.
2. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
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. 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. CA-125 has a low clinical sensitivity and specificity as a tumor marker. Clinically an elevated **CA-125 value alone is not of diagnostic value as a test for cancer** and should only be used in conjunction with other clinical manifestations (observations) and diagnostic parameters.

13.0 EXPECTED RANGE OF VALUES

The serum CA-125 is elevated in 1% of normal healthy women, 3% of normal healthy women with benign ovarian diseases and 6% of patients with non-neoplastic conditions (including but not limited to first trimester pregnancy, menstruation, endometriosis uterine fibrosis, acute salphingitis, hepatic diseases and inflammation of peritoneum or pericardium).

TABLE I
Expected Values for CA-125 AccuBind® ELISA Test System

Healthy and non-pregnant subjects	U≤U 35 U/ml

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

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

The within and between assay precisions of the CA-125 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 U/ml)

Sample	N	X	σ	C.V.
Level 1	20	3.1	0.22	7.1%
Level 2	20	28.0	1.42	5.0%
Level 3	20	161.2	4.21	2.6%

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

Sample	N	X	σ	C.V.
Level 1	10	3.7	0.44	11.8%
Level 2	10	25.3	1.81	7.1%
Level 3	10	154.0	5.11	3.4%

*As measured in ten experiments in duplicate.

14.2 Sensitivity

The CA-125 AccuBind® ELISA test system has a sensitivity of 1.0 U/ml. The sensitivity was ascertained by determining the variability of the '0' calibrator and using the 2σ (95% certainty) statistic to calculate the minimum dose.

14.3 Accuracy

The CA-125 AccuBind® ELISA test system was compared with a reference method. Biological specimens from low, normal, and elevated concentrations were assayed. The total number of such specimens was 121. The least square regression equation and the correlation coefficient were computed for CA-125 in comparison with the reference method. The data obtained is displayed in Table 4.

TABLE 4

Method	Mean	Least Square Regression Analysis	Correlation Coefficient
This Method (X)	5.67	y = -0.116 +1.032x	0.998
Reference (Y)	5.75		

14.4 Specificity

In order to test the specificity of the antibody pair used, massive concentrations of possible cross-reactants were added to known serum pools and assayed in parallel with the base sera. In addition some widely used, over-the-counter, drugs and some cytotoxic drugs (10 fold the normal dose) were tested in the assay. No cross reaction was found. Percent recoveries for some of these additions are listed below in Table 5.

TABLE 5

Analyte	Amount Added	% Recovery
Bilirubin	1 mMol/L	98 – 103%
Hemoglobin	1 mMol/L	100 – 106%
Triglycerides	10 mMol/L	96 – 110 %
RF	1000 kIU/L	97 – 107%
Biotin	25 µg/L	99 – 103%

15.0 REFERENCES

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Revision: 4 Date: 2019-Jul-16 DCO: 1353
MP3025 Product Code: *3025-300*

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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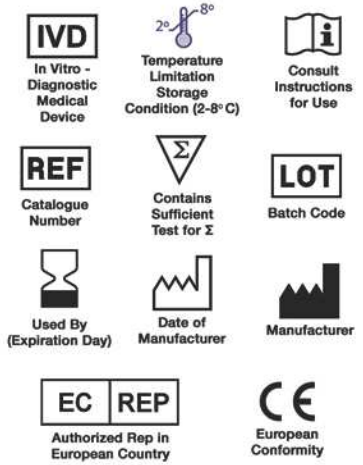
Glossary of Symbols
(EN 980/ISO 15223)



MULTI LIGAND CONTROL-TRI LEVEL
LOT# MLAC1E2

PRODUCT CODE: ML-300
EXP: 2025-05-18

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 should be assayed immediately after reconstitution, and 2) Folate and Insulin 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 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
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: 0 **Date: 2022-06-01** **Product Code: ML-300**

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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
Date of Manufacturer
LOT — Batch Code
Used By (Expiration Day)
Manufacturer
European Conformity
EC REP — Authorized Rep in European Country

DOCUMENT HISTORY

PREPARED BY: _____ DEPT: QC VERIFIED BY: AShatola DEPT: QA
APPROVED BY: _____ DEPT: Administration EFFECTIVE DATE: 2022-06-01
REVISION: 0 DCO: N/A

EXPECTED RANGE OF VALUES FOR MULTI-LIGAND CONTROL - TRI LEVEL
MASTER LOT:MLAC1E2

Analyte	A Range	B Range	C Range	Method
Allergy				
IgE in IU/ml	16.7 ± 5.51	214.17 ± 70.68	107 ± 35.31	MB ACCUBIND ELISA
	17.5 ± 5.78	230.2 ± 75.97	106.8 ± 35.24	MB ACCULITE CLIA
Anemia				
Ferritin in ng/ml	39.8 ± 13.13	76.7 ± 25.31	419.33 ± 138.38	MB ACCUBIND ELISA
	40.39 ± 13.33	76.14 ± 25.13	480.92 ± 158.70	MB ACCULITE CLIA
Vitamin B12 in pg/ml	299.15 ± 98.72	554.73 ± 183.06	1041.71 ± 343.77	MB ACCUBIND ELISA
	330.76 ± 109.15	560.33 ± 184.91	1106.86 ± 365.26	MB ACCULITE CLIA
Folate in ng/ml	2.27 ± 0.75	6.14 ± 2.03	9.09 ± 3.0	MB ACCUBIND ELISA
	2.74 ± 0.90	7.2 ± 2.38	9.91 ± 3.27	MB ACCULITE CLIA
Anemia Vast				
(Vitamin B12) in pg/ml	230.02 ± 75.91	517.05 ± 170.63	1013.01 ± 334.29	MB ACCUBIND ELISA
	241.24 ± 79.61	599.7 ± 197.9	860.18 ± 283.86	MB ACCULITE CLIA
(Folate) in ng/ml	2.27 ± 0.75	6.14 ± 2.03	9.09 ± 3.0	MB ACCUBIND ELISA
	2.74 ± 0.90	7.2 ± 2.38	9.91 ± 3.27	MB ACCULITE CLIA
Bone Metabolism				
Vit D Direct in ng/ml	21.05 ± 6.95	44.68 ± 14.75	98.05 ± 32.36	MB ACCUBIND ELISA
	22.05 ± 7.28	45.66 ± 15.07	115.94 ± 38.26	MB ACCULITE CLIA
Cancer Markers				
AFP in ng/ml	32 ± 10.56	115.3 ± 38.05	228.93 ± 75.55	MB ACCUBIND ELISA
	28 ± 9.24	108 ± 35.64	207 ± 68.31	MB ACCULITE CLIA
CEA in ng/ml	4.28 ± 1.41	15.24 ± 5.03	27.35 ± 9.03	MB ACCUBIND ELISA
	4.8 ± 1.58	17 ± 5.61	30 ± 9.90	MB ACCULITE CLIA
CEA Next Generation in ng/ml	4.18 ± 1.38	16.20 ± 5.35	29.46 ± 9.72	MB ACCUBIND ELISA
	4.09 ± 1.35	16.26 ± 5.36	30.32 ± 10.01	MB ACCULITE CLIA
fPSA in ng/ml	0.30 ± 0.10	1.81 ± 0.60	11.03 ± 3.64	MB ACCUBIND ELISA
	0.43 ± 0.14	1.98 ± 0.65	13.23 ± 4.46	MB ACCULITE CLIA
tPSA-XS in ng/ml	1.39 ± 0.46	3.03 ± 1.00	17.03 ± 5.62	MB ACCUBIND ELISA
	1.28 ± 0.42	3.01 ± 0.99	18.51± 6.11	MB ACCULITE CLIA
tPSA in ng/ml	2.97 ± 0.98	6.4 ± 2.11	36.3 ± 11.98	MB ACCUBIND ELISA
	2.27 ± 0.75	5.47 ± 1.81	33.33 ± 11.0	MB ACCULITE CLIA
Cancer Markers Vast				
(CEA) in ng/ml	4.45 ± 1.47	15.83 ± 5.22	26.17 ± 8.64	MB ACCUBIND ELISA
	4.26 ± 1.40	18.29 ± 6.04	27.98 ± 9.23	MB ACCULITE CLIA
(AFP) in ng/ml	25.12 ± 8.29	98.90 ± 32.64	192.94 ± 63.67	MB ACCUBIND ELISA
	25.18 ± 9.40	95.78 ± 31.61	183.31 ± 60.49	MB ACCULITE CLIA
(tPSA) in ng/ml	1.44 ± 0.48	4.23 ± 1.39	23.86 ± 7.87	MB ACCUBIND ELISA
	1.15 ± 0.38	3.90 ± 1.29	22.73 ± 7.50	MB ACCULITE CLIA
Cardiac Markers				
Dig in ng/ml	0.45 ± 0.15	1.55 ± 0.51	3.10 ± 1.02	MB ACCUBIND ELISA
	0.47 ± 0.15	1.51 ± 0.50	2.99 ± 0.99	MB ACCULITE CLIA
Diabetes				
C-Peptide in ng/ml	0.46 ± 0.15	2.8 ± 0.92	4.12 ± 1.36	MB ACCUBIND ELISA
	0.39 ± 0.13	2.97 ± 0.98	4.36 ± 1.44	MB ACCULITE CLIA
Insulin in µIU/ml	20.2 ± 6.67	48.59 ± 16.03	112.36 ± 37.08	MB ACCUBIND ELISA
	18.7 ± 6.17	51.7 ± 17.06	114.6 ± 37.82	MB ACCULITE CLIA
Rapid Insulin in µIU/ml	21.3 ± 7.03	48 ± 15.84	117 ± 38.61	MB ACCUBIND ELISA
Fertility				
FSH in mIU/ml	4.89 ± 1.61	24.53 ± 8.10	38.78 ± 12.80	MB ACCUBIND ELISA
	4.41 ± 1.46	23.78 ± 7.85	37.86 ± 12.49	MB ACCULITE CLIA
hCG in mIU/ml	6.08 ± 2.01	31.48 ± 10.39	164.71 ± 54.35	MB ACCUBIND ELISA
	4.5 ± 1.49	41.2 ± 13.60	217.4 ± 71.74	MB ACCULITE CLIA
hCG-XR in mIU/ml	4.40 ± 1.45	24.95 ± 8.23	158.90 ± 52.44	MB ACCUBIND ELISA
	3.89 ± 1.28	23.99 ± 7.92	147.32 ± 48.62	MB ACCULITE CLIA
LH in mIU/ml	4.43 ± 1.46	23.60 ± 7.79	51.47 ± 16.99	MB ACCUBIND ELISA
	4.18 ± 1.38	24.05 ± 7.94	51.63 ± 17.04	MB ACCULITE CLIA
PRL in ng/ml	5.48 ± 1.81	21.2 ± 7	73.16 ± 24.14	MB ACCUBIND ELISA
	4.4 ± 1.45	18.5 ± 6.11	66.6 ± 21.98	MB ACCULITE CLIA
PRL-seq in ng/ml	3.94 ± 1.49	13.17 ± 4.35	33.68 ± 11.11	MB ACCUBIND ELISA
	3.91 ± 1.29	12.79 ± 4.22	29.35 ± 9.68	MB ACCULITE CLIA
Rapid HCG in mIU/ml	6.05 ± 2.00	32.04 ± 10.57	166.16 ± 54.83	MB ACCUBIND ELISA
Fertility Vast				
(FSH) in mIU/ml	27.19 ± 8.97	115.80 ± 38.21	212.27 ± 70.05	MB ACCUBIND ELISA
	25.18 ± 8.31	110.00 ± 36.30	216.40 ± 71.41	MB ACCULITE CLIA
(LH) in mIU/ml	1.03 ± 0.34	2.90 ± 0.96	6.11 ± 2.01	MB ACCUBIND ELISA
	1.19 ± 0.39	2.58 ± 0.85	5.81 ± 1.92	MB ACCULITE CLIA
(hCG) in mIU/ml	4.05 ± 1.34	27.80 ± 9.17	139.0 ± 45.87	MB ACCUBIND ELISA
	4.74 ± 1.56	25.43 ± 8.39	141.98 ± 46.85	MB ACCULITE CLIA
Triple Screen VAST				
(AFP) in ng/ml	27.19 ± 8.97	115.80 ± 38.21	212.27 ± 70.05	MB ACCUBIND ELISA
	25.18 ± 8.31	110.00 ± 36.30	216.40 ± 71.41	MB ACCULITE CLIA
(uE3) in ng/ml	1.03 ± 0.34	2.90 ± 0.96	6.11 ± 2.01	MB ACCUBIND ELISA
	1.19 ± 0.39	2.58 ± 0.85	5.81 ± 1.92	MB ACCULITE CLIA
(hCG) in mIU/ml	4.05 ± 1.34	27.80 ± 9.17	139.0 ± 45.87	MB ACCUBIND ELISA
	4.74 ± 1.56	25.43 ± 8.39	141.98 ± 46.85	MB ACCULITE CLIA
Growth Deficiency				
hGH in µIU//ml	7.23 ± 2.39	26.1± 8.61	63.76 ± 21.04	MB ACCUBIND ELISA
	7 ± 2.31	28.2 ± 9.31	60.5 ± 19.97	MB ACCULITE CLIA
Steroids				
Cortisol in µg/dl	2.79 ± 0.92	15.30 ± 5.05	29.40 ± 9.70	MB ACCUBIND ELISA
	2.83 ± 0.94	13.64 ± 4.50	28.56 ± 9.42	MB ACCULITE CLIA
DHEA-S in µg/ml	0.34 ± 0.11	1.35± 0.45	4.38 ± 1.45	MB ACCUBIND ELISA
	0.44 ± 0.15	1.6 ± 0.53	4.5 ± 1.49	MB ACCULITE CLIA
DHEA in ng/ml	0.73 ± 0.33	4.03 ± 1.33	9.09 ± 3.0	MB ACCUBIND ELISA
	0.72 ± 0.24	4.58 ± 1.51	9.51 ± 3.14	MB ACCULITE CLIA
E2 in pg/ml	30.26 ± 9.98	169.49 ± 55.93	329.01 ± 108.57	MB ACCUBIND ELISA
	28.75 ± 9.49	171.0 ± 56.43	348.85 ± 115.12	MB ACCULITE CLIA
Progesterone in ng/ml	1.1± 0.36	8.44 ± 2.79	24.59 ± 8.11	MB ACCUBIND ELISA
	1.4 ± 0.46	10 ± 3.30	26.5 ± 8.75	MB ACCULITE CLIA
17-OHP in ng/ml	0.50 ± 0.17	2.09 ± 0.69	5.33 ± 1.87	MB ACCUBIND ELISA
	0.55 ± 0.18	2.14 ± 0.71	5.34 ± 1.76	MB ACCULITE CLIA
17-OHP-SI in ng/ml	0.35 ± 0.12	1.13 ± 0.37	3.15 ± 1.04	MB ACCUBIND ELISA
	0.31 ± 0.10	1.33 ± 0.44	3.66 ± 1.21	MB ACCULITE CLIA
Testosterone in ng/ml	0.29 ± 0.09	1.21 ± 0.40	6.62 ± 2.18	MB ACCUBIND ELISA
	0.37 ± 0.12	1.28 ± 0.42	7.34 ± 2.42	MB ACCULITE CLIA
uE3 in ng/ml	1.13 ± 0.42	2.40 ± 0.79	6.27 ± 2.07	MB ACCUBIND ELISA
	1.15 ± 0.38	2.51 ± 0.83	5.78 ± 1.91	MB ACCULITE CLIA
E1 in ng/ml	42.69 ± 14.09	191.59 ± 63.22	508.55 ± 167.82	MB ACCUBIND ELISA
	43.68 ± 14.41	213.38 ± 70.42	488.08 ± 161.06	MB ACCULITE CLIA
ANST in ng/ml	0.32 ± 0.11	0.86 ± 0.28	7.1 ± 2.34	MB ACCUBIND ELISA
Aldosterone in ng/ml	85.84 ± 28.33	342.19 ± 112.92	1011.35 ± 333.75	MB ACCUBIND ELISA
	86.9 ± 28.68	401.2 ± 132.40	1112.74 ± 367.20	MB ACCULITE CLIA
Free Testosterone (0-60pg/ml calibration)	0.93 ± 0.31	2.63 ± 0.87	20.29 ± 6.70	MB ACCUBIND ELISA
	0.97 ± 0.32	2.66 ± 0.88	27.24 ± 8.99	MB ACCULITE CLIA
Thyroid				
T3 in ng/ml	0.51 ± 0.17	1.35 ± 0.45	3.43 ± 1.13	MB ACCUBIND ELISA
	0.59 ± 0.24	1.43 ± 0.47	3.27 ± 1.08	MB ACCULITE CLIA
T4 in µg/dl	2.82 ± 0.93	6.54 ± 2.16	16.48 ± 5.44	MB ACCUBIND ELISA
	2.75 ± 0.91	6.60 ± 2.18	15.33 ± 5.06	MB ACCULITE CLIA
TSH in µIU/ml	0.40 ± 0.13	4.00 + 1.32	20.22 ± 6.67	MB ACCUBIND ELISA
	0.42 ± 0.14	4.00 ± 1.32	21.39 ± 7.06	MB ACCULITE CLIA
fT3 in pg/ml	2.01 ± 0.66	4.11 ± 1.36	8.53 ± 2.81	MB ACCUBIND ELISA
	2.24 ± 0.74	4.12 ± 1.36	7.98 ± 2.63	MB ACCULITE CLIA
fT4 in ng/dl	0.52 ± 0.17	1.28 ± 0.42	4.06 ± 1.34	MB ACCUBIND ELISA
	0.54 ± 0.18	1.19 ± 0.39	3.83 ± 1.26	MB ACCULITE CLIA
T3-Uptake in %U	32.35 ± 1.86	30.65 ± 1.86	46.15 ± 1.85	MB ACCUBIND ELISA
	31.55 ± 2.42	30.88 ± 2.34	46.55 ± 2.70	MB ACCULITE CLIA
Rapid TSH in µIU/ml	0.59 ± 0.20	4.17 ± 1.37	19.63 ± 6..48	MB ACCUBIND ELISA
	0.31 ± 0.12	4.05 ± 1.34	21.0 ± 6.93	MB ACCULITE CLIA
TSH-RC in µIU/ml	0.56 ± 0.18	4.61 ± 1.52	21.88 ± 7.22	MB ACCUBIND ELISA
Thyroid VAST				
(TSH) in µIU/ml	0.37 ± 0.12	4.18 ± 1.38	22.95 ± 7.57	MB ACCUBIND ELISA
	0.31 ± 0.10	4.32 ± 1.43	24.24 ± 8.00	MB ACCULITE CLIA
Strep T3 in ng/ml	0.51 ± 0.17	1.46 ± 0.48	3.15 ± 1.04	MB ACCUBIND ELISA
	0.47 ± 0.16	1.32 ± 0.43	3.14 ± 1.04	MB ACCULITE CLIA
Strep T4 in µg/dl	3.27 ± 1.08	8.54 ± 2.82	17.19 ± 5.67	MB ACCUBIND ELISA
	2.98 ± 0.98	8.26 ± 2.73	16.29 ± 5.37	MB ACCULITE CLIA
Free Thyroid VAST				
(TSH) in µIU/ml	0.46 ± 0.15	4.64 ± 1.53	23.78 ± 7.85	MB ACCUBIND ELISA
	0.53 ± 0.17	4.87 ± 1.61	22.56 ± 7.44	MB ACCULITE CLIA
Strept fT3 in pg/ml	1.81 ± 0.60	3.42 ± 1.13	9.78 ± 4.20	MB ACCUBIND ELISA
	1.79 ± 0.59	3.87 ± 1.28	8.64 ± 2.85	MB ACCULITE CLIA
Strept fT4 in ng/dl	0.91 ± 0.30	1.25 ± 0.41	5.22 ± 1.72	MB ACCUBIND ELISA
	0.94 ± 0.31	1.26 ± 0.41	5.32 ± 1.75	MB ACCULITE CLIA

Tumor Marker Control **PRODUCT CODE: TMC-300**

LOT# TMCAC1K1 **EXP: 2024/11/30**

INTENDED USE

The Tumor Marker 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, liquid 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 The Tumor Marker Controls are intended to be used in the exact manner as patient samples. The control is packaged as 6 vials of 2.0 ml. 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

This control is provided liquid and ready to use. This product will be stable until the expiration date when stored unopened at <-20°C. Once the control is opened, all analytes will be stable for 7 days when stored tightly capped at 2 to 8°C. To avoid contamination, it is recommended labs aliquot required quantities into vials before each use.

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.) 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	< -20°C
Unopened	Ninety (90) days	2 – 8°C
Opened	Seven (7) days	2 – 8°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.

Analyte	A Range	B Range	C Range	Method
CA 125 in U/ml	15.69 ± 5.18	60.31 ± 19.90	117.81 ± 38.88	MB ACCUBIND ELISA
	18.16 ± 5.99	64.25 ± 21.20	126.93 ± 41.89	MB ACCULITE CLIA
CA 19-9 in U/ml	14.89 ± 5.43	52.19 ± 17.22	91.50 ± 30.19	MB ACCUBIND ELISA
	15.01 ± 5.08	46.89 ± 15.47	80.73 ± 26.64	MB ACCULITE CLIA
CA 15-3 in U/ml	15.50 ± 5.12	47.47 ± 15.67	93.09 ± 30.72	MB ACCUBIND ELISA
	14.63 ± 4.83	44.10 ± 19.09	105.29 ± 34.75	MB ACCULITE CLIA

Individual laboratory means should fall within the corresponding acceptable range; however laboratory means may vary from the listed values during the life of this control. Therefore, each laboratory should establish its own means and acceptable ranges for the product used, using Monobind's assignment only as guide. A trend log should be maintained for batch to batch consistency of the test. Variations over time and between laboratories may be caused by a) differences in laboratory personnel, b) improper technique, c) instrumentation and reagents, d) improper dilutions from the stated manufacturer's procedure, and/ or e) modifications in the manufacturer's test procedure.

Refer to http://www.monobind.com/site/qc-documents.html for any updated insert information.

WARNING AND PRECAUTIONS

FOR IN VITRO DIAGNOSTIC USE

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

Revision: 0 **Date: 2021-NOV-30** **Product Code: TMC-300**

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IVD

EC REP **CEpartner4U**, Esdoornlaan 13, 3951DB Maarn, The Netherlands www.cepartner4u.eu

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
Date of Manufacturer
LOT — Batch Code
Used By (Expiration Day)
Manufacturer
European Conformity
EC REP — Authorized Rep in European Country

DOCUMENT HISTORY

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