



**β-Human Chorionic Gonadotropin (hCG)
Test System
Product Code: 825-300**

1.0 INTRODUCTION

Intended Use: The Quantitative Determination of Chorionic Gonadotropin (hCG) Concentration in Human Serum by a Microplate Enzyme Immunoassay, Colorimetric

2.0 SUMMARY AND EXPLANATION OF THE TEST

Human chorionic gonadotropin (hCG) concentration increases dramatically in blood and urine during normal pregnancy. hCG is secreted by placental tissue, beginning with the primitive trophoblast, almost from the time of implantation, and serves to support the corpus luteum during the early weeks of pregnancy. hCG or hCG similar glycoproteins can also be produced by a wide variety of trophoblastic and nontrophoblastic tumors. The measurement of hCG, by assay systems with suitable sensitivity and specificity has proven great value in the detection of pregnancy and the diagnosis of early pregnancy disorders.

According to the literature, hCG is detectable as early as 10 days after ovulation, reaching 100 mIU/ml by the first missed period. At the time for the next ovulation, the hCG level is 200 mIU/ml (approximately 28 days after conception).¹ A peak of 50,000 or even 100,000 mIU/ml is attained by the third month, then a gradual decline is observed.^{2,3}

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

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

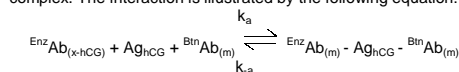
The employment of several serum references of known chorionic gonadotropin levels permits construction of a dose response curve of activity and concentration. From comparison to the dose response curve, an unknown specimen's activity can be correlated with chorionic gonadotropin concentration.

3.0 PRINCIPLE

Immunoenzymometric assay (TYPE 3):

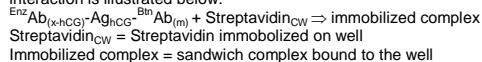
The essential reagents required for an immunoenzymometric assay include high affinity and specificity antibodies (enzyme and immobilized), with different and distinct epitope recognition, in excess, and native antigen. In this procedure, the immobilization takes place during the assay at the surface of a microplate well

through the interaction of streptavidin coated on the well and exogenously added biotinylated monoclonal anti-hCG antibody. Upon mixing monoclonal biotinylated antibody, the enzyme-labeled antibody and a serum containing the native antigen, reaction results between the native antigen and the antibodies without competition or steric hindrance to form a soluble sandwich complex. The interaction is illustrated by the following equation:



$\text{Biotin Ab}_{(m)}$ = Biotinylated Monoclonal Antibody (Excess Quantity)
 Ag_{hCG} = Native Antigen (Variable Quantity)
 $\text{Enz Ab}_{(x\text{-hCG})}$ = Enzyme labeled Antibody (Excess Quantity)
 $\text{Enz Ab}_{(m)} - \text{Ag}_{\text{hCG}} - \text{Biotin Ab}_{(m)}$ = Ag-Antibodies Sandwich complex
 k_a = Rate Constant of Association
 k_a = Rate Constant of Dissociation

Simultaneously, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antibody. This interaction is illustrated below:



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

4.0 REAGENTS

Materials Provided:

- A. hCG Calibrators – 1 ml/vial - Icons A-F**
Six (6) vials of references for hCG Antigen at levels of 0(A), 5(B), 25(C), 50(D), 100(E) and 250(F) mIU/ml. Store at 2-8°C. A preservative has been added.
Note: The calibrators, human serum based, were calibrated using a reference preparation, which was assayed against the WHO 3rd IS (75/537).
- B. hCG Enzyme Reagent – 13 ml/vial - Icon**
One (1) vial containing enzyme labeled affinity purified antibody, biotinylated monoclonal mouse IgG in buffer, dye, and preservative. Store at 2-8°C.
- C. Streptavidin Coated Plate – 96 wells – Icon**
One 96-well microplate coated with streptavidin and packaged in an aluminum bag with a drying agent. Store at 2-8°C.
- D. Wash Solution Concentrate – 20 ml/vial - Icon**
One (1) vial containing a surfactant in buffered saline. A preservative has been added. Store at 2-8°C.
- E. Substrate A – 7ml/vial - Icon S^A**
One (1) vial containing tetramethylbenzidine (TMB) in buffer. Store at 2-8°C.
- F. Substrate B – 7ml/vial - Icon S^B**
One (1) vial containing hydrogen peroxide (H₂O₂) in buffer. Store at 2-8°C.
- G. Stop Solution – 8ml/vial - Icon**
One (1) vial containing a strong acid (1N HCl). Store at 2-8°C.

H. Product Instructions.

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

4.1 Required But Not Provided:

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

8. Timer.
9. Quality control materials

5.0 PRECAUTIONS

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

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

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

6.0 SPECIMEN COLLECTION AND PREPARATION

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

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

Samples may be refrigerated at 2-8°C for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20°C for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.05 ml (50µl) of the specimen is required.

7.0 QUALITY CONTROL

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

8.0 REAGENT PREPARATION

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

**Note1: Do not use the working substrate if it looks blue.
Note 2: Do not use reagents that are contaminated or have bacteria growth.**

9.0 TEST PROCEDURE

Before proceeding with the assay, bring all reagents, serum reference calibrators and controls to room temperature (20-27°C).

****Test Procedure should be performed by a skilled individual or trained professional****

1. Format the microplate wells for each serum reference calibrator, control and patient specimen to be assayed in duplicate. **Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C**

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

DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION

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

10.0 CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of Human chorionic gonadotropin (hCG) in unknown specimens.

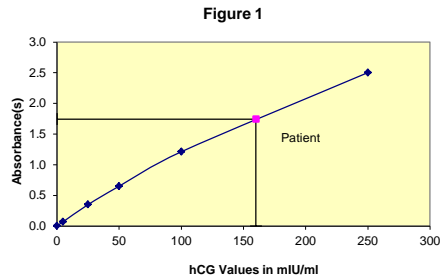
1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the absorbance for each duplicate serum reference versus the corresponding hCG concentration in mIU/ml on linear graph paper (do not average the duplicates of the serum references before plotting).
3. Draw the best-fit curve through the plotted points.
4. To determine the concentration of hCG for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in mIU/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance (1.745) intersects the dose response curve at (157 mIU/ml) hCG concentration (See Figure 1).

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

EXAMPLE 1

Sample I.D.	Well Number	Abs (A)	Mean Abs (B)	Value (mIU/ml)
Cal A	A1	0.002	0.004	0
	B1	0.005		
Cal B	C1	0.073	0.071	5
	D1	0.069		
Cal C	E1	0.340	0.350	25
	F1	0.360		
Cal D	G1	0.637	0.650	50
	H1	0.663		
Cal E	A2	1.223	1.212	100
	B2	1.199		
Cal F	C2	2.518	2.502	250
	D2	2.486		
Ctrl 1	E2	0.075	0.076	5.8
	F2	0.077		
Ctrl 2	G2	0.280	0.290	21.9
	H2	0.301		
Patient	A3	1.736	1.745	157
	B3	1.754		

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



11.0 Q.C. PARAMETERS

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

- The absorbance (OD) of calibrator 'F' should be ≥ 1.3 .
- 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

- It is important that the time of reaction in each well is held constant to achieve reproducible results.
- Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
- Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
- If more than one (1) plate is used, it is recommended to repeat the dose response curve.
- 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.
- Plate readers measure vertically. Do not touch the bottom of the wells.
- Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
- Use components from the same lot. No intermixing of reagents from different batches.
- Patient specimens with hCG concentrations above 250 mIU/ml may be diluted with normal male serum (hCG < 1 mIU/ml) and re-assayed. The sample's concentration is obtained by multiplying the result by the dilution factor.
- 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.
- 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.
- 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.
- 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

- Measurements and interpretation of results must be performed by a skilled individual or trained professional.**
- 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.
- The reagents for the test system have been formulated to eliminate maximal interference; however, potential interaction between rare serum specimens and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for all kinds of immunoassays (Boscato LM, Stuart MC. 'Heterophilic antibodies: a problem for all immunoassays' Clin. Chem. 1988:3427-33). For diagnostic purposes, the results from this assay should be in combination with clinical examination, patient history and all other clinical findings. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.

- 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.**
- 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.
- False positive results may occur in the presence of a wide variety of trophoblastic and nontrophoblastic tumors that secrete hCG. Therefore, the possibility of an hCG secreting neoplasia should be eliminated prior to diagnosing pregnancy.
- Also, false positive results may be seen when assaying specimens from individuals taking the drugs Pergonal* and Clomid**. Additionally Pergonal will often be followed with an injection of hCG.
- Spontaneous microabortions and ectopic pregnancies will tend to have values which are lower than expected during a normal pregnancy while somewhat higher values are often seen in multiple pregnancies.^{5,6,7}
- Following therapeutic abortion, detectable hCG may persist for as long as three to four weeks. The disappearance rate of hCG, after spontaneous abortion, will vary depending upon the quantity of viable residual trophoblast.^{4,5,6,7}
- A hCG value alone is not of diagnostic value** and should only be used in conjunction with other clinical manifestations (observations) and diagnostic procedures.

*Pegonal is a registered trademark of Serono Laboratories, Inc.
 **Clomid is a registered trademark of Merriell-National Laboratories

13.0 EXPECTED RANGES OF VALUES

A study of an apparent normal adult population was undertaken to determine expected values for the HCG AccuBind® ELISA Test System. The mean (X) values, standard deviations (σ) and expected ranges ($\pm 2\sigma$) are presented in Table 1.

TABLE 1
Expected Values for the hCG ELISA Test System
(In mIU/ml - 3rd IS 75/537)

Number	25
Mean	2.9
Standard Deviation	1.4
Expected Ranges ($\pm 2\sigma$)	0.1 - 5.7

Expected levels for hCG during normal pregnancy (3) are listed in Table 2.

TABLE 2
Expected Values for hCG Levels (3rd IS 75/537)
during normal pregnancy (in mIU/ml)

1 st week	10 - 30
2 nd week	30 - 100
3 rd week	100 - 1000
4 th week	1,000 - 10,000
2 nd & 3 rd month	30,000 - 100,000
2 nd trimester	10,000 - 30,000
3 rd trimester	5,000 - 15,000

Values for hCG for a normal, healthy population and pregnant women, during gestation cycle, are given in Table 3. The values depicted below represent limited in house studies in concordance with published literature.^{8,9,10}

TABLE 3
Median Values during Gestation.

Gestation (Week)	hCG (IU/ml)
15	40.88
16	33.87
17	28.71
18	26.74
19	18.76
20	19.24
21	23.46

It is important to keep in mind that establishment of a range of values which can be expected to be found by a given method for a population of "normal"-persons is dependent upon a multiplicity of factors: the specificity of the method, the population tested and

the precision of the method in the hands of the analyst. For these reasons each laboratory should depend upon the range of expected values established by the Manufacturer only until an in-house range can be determined by the analysts using the method with a population indigenous to the area in which the laboratory is located.

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

The within and between assay precisions of the hCG AccuBind® ELISA were determined by analyses on three different levels of control sera. The number (N), mean value (X), standard deviation (σ) and coefficient of variation (C.V.) for each of these control sera are presented in Table 4 and Table 5.

TABLE 4
Within Assay Precision (Values in mIU/ml)

Sample	N	X	σ	C.V.
Level 1	20	4.4	0.22	4.9%
Level 2	20	18.7	0.75	4.0%
Level 3	20	214.8	14.59	6.8%

TABLE 5
Between Assay Precision* (Values in mIU/ml)

Sample	N	X	σ	C.V.
Level 1	20	5.4	0.52	9.6%
Level 2	20	22.4	1.97	8.8%
Level 3	20	213.1	15.16	7.1%

*As measured in ten experiments in duplicate.

14.2 Sensitivity

The hCG AccuBind® ELISA test system has a sensitivity of 0.003 mIU/well. This is equivalent to a sample containing 0.102 mIU/ml hCG concentration. The analytical sensitivity (detection limit) was ascertained by determining the variability of the '0 mIU/ml' calibrator and using the 2 σ (95% certainty) statistic to calculate the minimum dose.

14.3 Accuracy

This hCG AccuBind® ELISA test system was compared with a reference radioimmunoassay. Biological specimens from normal and pregnant populations were assayed. The total number of such specimens was 110. The least square regression equation and the correlation coefficient were computed for the hCG ELISA in comparison with the reference method. The data obtained is displayed below.

TABLE 6

Method	Mean (x)	Least Square Regression Analysis	Correlation Coefficient
Monobind	14.8	y = 0.081 + 0.93(x)	0.989
Reference	15.1		

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

14.4 Specificity

The cross-reactivity of the hCG AccuBind® ELISA to selected substances was evaluated by adding the interfering substance to a serum matrix at various concentrations. The cross-reactivity was calculated by deriving a ratio between dose of interfering substance to dose of chorionic gonadotropin needed to produce the same absorbance.

Substance	Cross Reactivity	Concentration
Chorionic Gonadotropin (hCG)	1.0000	----
β -hCG subunit	< 0.0001	1000ng/ml
Follitropin (FSH)	< 0.0001	1000ng/ml
Lutropin Hormone (LH)	< 0.0001	1000ng/ml
hrotropin (TSH)	< 0.0001	1000ng/ml

14.5 Hook Effect

The test shows no hook effect up to concentrations of > 150,000 mIU/ml.

15.0 REFERENCES

- Kosasa TS, "Measurement of Human Chorionic Gonadotropin", *Journal of Reproductive Medicine*, 26, 201-6 (1981).
- Danzer H, Braunstein GD, et al, "Maternal Serum Human Chorionic Gonadotropin Concentrations and Fetal Sex Predictions", *Fertility and Sterility*, 34, 336-40 (1980).
- Braunstein G.D., et al., "Serum Human Chorionic Gonadotropin Levels through Normal Pregnancy", *American Journal of Obstetrics and Gynecology* 126, pg. 678-81 (1976).
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- Braunstein GD, et al, "First-Trimester Chorionic Gonadotropin Measurements as an Aid to the Diagnosis of Early Pregnancy Disorders", *American Journal of Obstetrics and Gynecology*, 131, 25-32 (1978).
- Lenton E, Neal L and Sulaiman R, "Plasma Concentrations of Human Gonadotropin from the time of Implantation until the Second Week of Pregnancy", *Fertility and Sterility*, 37, 773-78 (1982).
- Canick JA, Rish S. 'The accuracy of assigned risks in maternal serum screening', *Prenatal Diagnosis*; 18:413-415 (1998).
- NIH State-of-the Science Conference Statement on Management of Menopause-Related Symptoms. NIH Consensus State Sci Statements. Mar 21-23; 22(1), 1-38 (2005).
- Tietz NW, Ed: *Clinical Guide to Laboratory Tests* 3rd Ed, Philadelphia, WA Saunders Co (1995).

Revision: 5 Date: 2021-Sep-23 DCO: 1509
 MP825 Product Code: 825-300

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

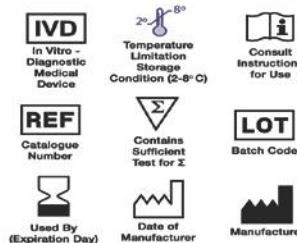
For Orders and Inquiries, please contact
Monobind Inc.
 100 North Pointe Drive
 Lake Forest, CA 92530 USA

Tel: +1 949.951.2665 Mail: info@monobind.com
 Fax: +1 949.951.3539 Fax: www.monobind.com



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

Glossary of Symbols
 (EN 960/ISO 15223)





Follicle Stimulating Hormone (FSH) Test System Product Code: 425-300

1.0 INTRODUCTION

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

2.0 SUMMARY AND EXPLANATION OF THE TEST

Follicle Stimulating hormone (FSH) is a glycoprotein consisting of two subunits with an approximate molecular mass of 35,500 daltons. The α -subunit is similar to other pituitary hormones [lutetizing stimulating hormone (LH), thyroid stimulating hormone (TSH) and chorionic gonadotropin (CG)] while the β -subunit is unique. The β -subunit confers the biological activity to the molecule. Stimulation by gonadotropin-releasing hormone (GnRH) causes release of FSH, as well as LH, from the pituitary and is transported by the blood to their sites of action, the testes or ovary.

In men, FSH acts on the Sertoli cells of the testis, stimulating the synthesis of inhibin, which appears to specifically inhibit further FSH secretion, and androgen-binding protein. Thus, it indirectly supports spermatogenesis. In women, FSH acts on the granulosa cells of the ovary, stimulating steroidogenesis. All ovulatory menstrual cycles have a characteristic pattern of FSH, as well as LH, secretion. The menstrual cycle is divided into a follicular phase and a luteal phase by the midcycle surge of the gonadotropins (LH and FSH). As the follicular phase progresses, FSH concentration decreases. Near the time ovulation occurs, about midcycle, FSH peaks (lesser in magnitude than LH) to its highest level.

The clinical usefulness of the measurement of Follicle Stimulating hormone (FSH) in ascertaining the homeostasis of fertility regulation via the hypothalamic - pituitary - gonadal axis has been well established.^{1,2}

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

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

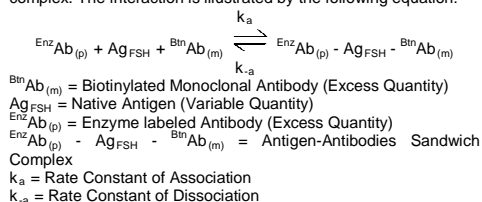
The employment of several serum references of known Follicle Stimulating Hormone levels permits construction of a dose response curve of activity and concentration. From comparison to

the dose response curve, an unknown specimen's activity can be correlated with Follicle Stimulating Hormone concentration.

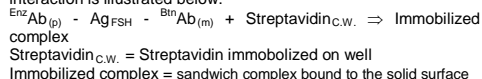
3.0 PRINCIPLE

Immunoenzymometric assay (TYPE 3):

The essential reagents required for an immunoenzymometric assay include high affinity and specificity antibodies (enzyme and immobilized), with different and distinct epitope recognition, in excess, and native antigen. In this procedure, the immobilization takes place during the assay at the surface of a microplate well through the interaction of streptavidin coated on the well and exogenously added biotinylated monoclonal anti-FSH antibody. Upon mixing monoclonal biotinylated antibody, the enzyme-labeled antibody and a serum containing the native antigen, reaction results between the native antigen and the antibodies without competition or steric hindrance to form a soluble sandwich complex. The interaction is illustrated by the following equation:



Simultaneously, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antibody. This interaction is illustrated below:



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

4.0 REAGENTS

Materials Provided:

A. FSH Calibrators – 1 ml/vial - Icons A-F

Six (6) vials of references for FSH Antigen at levels of 0(A), 5(B), 10(C), 25(D), 50(E) and 100(F) mIU/ml. Store at 2-8°C. A preservative has been added.

Note: The calibrators, human serum based, were calibrated using a reference preparation, which was assayed against the WHO 2nd IRP (78/549).

B. FSH Enzyme Reagent – 13 ml/vial - Icon

One (1) vial-containing enzyme labeled antibody, biotinylated monoclonal mouse IgG in buffer, dye, and preservative. Store at 2-8°C.

C. Streptavidin Coated Plate – 96 wells - Icon

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

D. Wash Solution Concentrate – 20 ml/vial - Icon

One (1) vial containing a surfactant in buffered saline. A preservative has been added. Store at 2-8°C.

E. Substrate A – 7.0ml/vial - Icon S^A

One (1) vial containing tetramethylbenzidine (TMB) in buffer. Store at 2-8°C.

F. Substrate B – 7.0ml/vial - Icon S^B

One (1) vial containing hydrogen peroxide (H₂O₂) in buffer. Store at 2-8°C.

G. Stop Solution – 8ml/vial - Icon

One (1) vial containing a strong acid (1N HCl). Store at 2-8°C.

H. Product Instructions.

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

Note 2: Avoid extended exposure to heat and light. **Opened reagents are stable for sixty (60) days when stored at**

2-8°C. Kit and component stability are identified on the label.

Note 3: Above reagents are for a single 96-well microplate.

4.1 Required But Not Provided:

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

5.0 PRECAUTIONS

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

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

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

6.0 SPECIMEN COLLECTION AND PREPARATION

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

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

Samples may be refrigerated at 2-8°C for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20°C for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.100ml (100µl) of the specimen is required.

7.0 QUALITY CONTROL

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

8.0 REAGENT PREPARATION

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

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

10.0 CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of follicle stimulating hormone in unknown specimens.

1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the absorbance for each duplicate serum reference versus the corresponding FSH concentration in mIU/ml on linear graph paper (do not average the duplicates of the serum references before plotting).
3. Draw the best-fit curve through the plotted points.
4. To determine the concentration of FSH for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in mIU/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance (1.214) intersects the dose response curve at 43.2mIU/ml FSH concentration (See Figure 1).

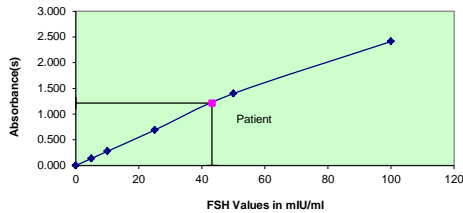
Note: Computer data reduction software designed for ELISA assays may also be used for the data reduction. **If such software is utilized, the validation of the software should be ascertained.**

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

EXAMPLE 1

Sample I.D.	Well Number	Abs (A)	Mean Abs (B)	Value (mIU/ml)
Cal A	A1	0.001	0.001	0
	B1	0.001		
Cal B	C1	0.146	0.139	5
	D1	0.133		
Cal C	E1	0.276	0.277	10
	F1	0.278		
Cal D	G1	0.680	0.689	25
	H1	0.698		
Cal E	A2	1.444	1.399	50
	B2	1.354		
Cal F	C2	2.471	2.412	100
	D2	2.354		
Ctrl 1	E2	0.162	0.157	5.6
	F2	0.152		
Ctrl 2	G2	0.545	0.546	19.9
	H2	0.547		
Patient	A3	1.173	1.214	43.2
	B3	1.255		

Figure 1



11.0 Q.C. PARAMETERS

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

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

12.0 RISK ANALYSIS

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

12.1 Assay Performance

- It is important that the time of reaction in each well is held constant to achieve reproducible results.
- Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
- Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
- If more than one (1) plate is used, it is recommended to repeat the dose response curve.
- 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.
- Plate readers measure vertically. Do not touch the bottom of the wells.
- Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
- Use components from the same lot. No intermixing of reagents from different batches.
- 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.
- 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.

- 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.
- 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

- Measurements and interpretation of results must be performed by a skilled individual or trained professional.**
- 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.
- 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;34:27-33). For diagnostic purposes, the results from this assay should be in combination with clinical examination, patient history and all other clinical findings. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
- 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.
- 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.
- FSH is suppressed by estrogen but in woman taking oral contraceptives the level may be low or normal. Excessive dieting and weight loss may lead to low gonadotropin concentrations.
- Follicle Stimulating Hormones are dependent upon diverse factors other than pituitary homeostasis. Thus, the determination alone is not sufficient to assess clinical status.

13.0 EXPECTED RANGES OF VALUES

A study of an apparent normal adult population was undertaken to determine expected values for the FSH Accubind® ELISA Test System. The expected values are presented in Table 1.

TABLE 1
Expected Values for the FSH Accubind® ELISA Test System (in mIU/ml 2nd IRP 78/549)

Women		
Follicular phase	3.0 --	12.0
Midcycle	8.0 --	22.0
Luteal phase	2.0 --	12.0
Postmenopausal	35.0 --	151.0
Men		
	1.0 --	14.0

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

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

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

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

Sample	N	X	σ	C.V.
Level 1	20	5.0	0.25	5.4%

Level 2	20	25.0	0.94	3.8%
Level 3	20	40.6	1.64	4.0%

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

Sample	N	X	σ	C.V.
Level 1	20	4.7	0.42	9.0%
Level 2	20	23.1	1.99	8.6%
Level 3	20	37.8	3.2	8.4%

*As measured in ten experiments in duplicate.

14.2 Sensitivity

The Follicle Stimulating Hormone procedure has a sensitivity of 0.006 mIU/well. This is equivalent to a sample containing 0.134mIU/ml FSH concentration. The sensitivity (detection limit) was ascertained by determining the variability of the '0 mIU/ml' calibrator and using the 2σ (95% certainty) statistic to calculate the minimum dose.

14.3 Accuracy

This FSH Accubind® ELISA test system was compared with a reference radioimmunoassay. Biological specimens from low, normal, and elevated concentrations were assayed. The total number of such specimens was 106. The least square regression equation and the correlation coefficient were computed for the FSH Accubind® ELISA test system in comparison with the reference method. The data obtained is displayed in Table 4.

TABLE 4

Method	Mean (x)	Least Square Regression Analysis	Correlation Coefficient
Monobind	17.4	$y = 0.98(x) - 1.7$	0.978
Reference	19.5		

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

14.4 Specificity

The cross-reactivity of the FSH Accubind® ELISA test system to selected substances was evaluated by adding the interfering substance to a serum matrix at various concentrations. The cross-reactivity was calculated by deriving a ratio between dose of interfering substance to dose of Follicle Stimulating Hormone needed to produce the same absorbance.

Substance	Cross Reactivity	Concentration
Follitropin (FSH)	1.0000	--
Lutropin Hormone (hLH)	< 0.0001	1000ng/ml
Chorionic Gonadotropin (hCG)	< 0.0001	1000ng/ml
Thyrotropin (TSH)	< 0.0001	1000ng/ml

15.0 REFERENCES

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Revision: 4 Date: 2019-Jul-16 DCO: 1353
MP425 Cat #: 425-300

Size	96(A)	192(B)	
	A)	1ml set	1ml set
Reagent (fill)	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)

For Orders and Inquires, please contact

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

Tel: +1 949.951.2665 Mail: info@monobind.com
Fax: +1 949.951.3539 Fax: www.monobind.com



CEpartner4U, Edoosdlaan 13
3951 DBMaarn, The Netherlands
www.cepartner4u.eu

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Glossary of Symbols
(EN 980/ISO 15223)



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

1.0 INTRODUCTION

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

2.0 SUMMARY AND EXPLANATION OF THE TEST

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

The clinical usefulness of the measurement of luteinizing hormone (LH) in ascertaining the homeostasis of fertility regulation via the hypothalamic - pituitary - gonadal axis has been well established.^{1,2} In addition, the advent of *in vitro* fertilization (IVF) technology to overcome infertility-associated problems has provided the impetus for rapid improvement in LH assay methodology from the technically demanding bioassay³ to the procedurally simple and rapid immunoenzymometric assays.

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

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

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

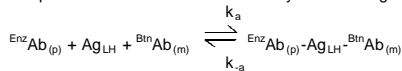
3.0 PRINCIPLE

Immunoenzymometric assay (TYPE 3):

The essential reagents required for an immunoenzymometric assay include high affinity and specificity antibodies (enzyme and immobilized), with different and distinct epitope recognition, in

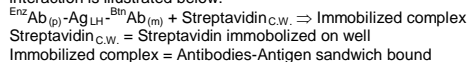
excess, and native antigen. In this procedure, the immobilization takes place during the assay at the surface of a microplate well through the interaction of streptavidin coated on the well and exogenously added biotinylated monoclonal anti-LH antibody.

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



$\text{Biot}^{\text{Ab}}_{(m)}$ = Biotinylated Monoclonal Antibody (Excess Quantity)
 Ag_{LH} = Native Antigen (Variable Quantity)
 $\text{Enz}^{\text{Ab}}_{(p)}$ = Enzyme labeled Antibody (Excess Quantity)
 $\text{Enz}^{\text{Ab}}_{(p)}\text{-Ag}_{\text{LH}}\text{-Biot}^{\text{Ab}}_{(m)}$ = 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:



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

4.0 REAGENTS

Materials Provided

A. LH Calibrators – 1ml/vial – Icons A-F

Six (6) vials of references for LH Antigen at levels of 0(A), 5(B), 25(C), 50(D), 100(E) and 200(F) mIU/ml. Store at 2-8°C. A preservative has been added.

Note: The calibrators, human serum based, were calibrated using a reference preparation, which was assayed against the WHO 2nd IS 80/552.

B. LH Enzyme Reagent – 13 ml/vial – Icon

One (1) vial containing enzyme labeled affinity purified antibody, biotinylated monoclonal mouse IgG in buffer, dye, and preservative. Store at 2-8°C.

C. Streptavidin Coated Plate – 96 wells – Icon

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

D. Wash Solution Concentrate – 20 ml/vial - Icon

One (1) vial containing a surfactant in buffered saline. A preservative has been added. Store at 2-8°C.

E. Substrate A – 7ml/vial – Icon

One (1) vial containing tetramethylbenzidine (TMB) in buffer. Store at 2-8°C.

F. Substrate B – 7ml/vial – Icon

One (1) vial containing hydrogen peroxide (H₂O₂) in buffer. Store at 2-8°C.

G. Stop Solution – 8ml/vial – Icon

One (1) vial containing a strong acid (1N HCl). Store at 2-8°C.

H. Product Instructions.

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

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

Note 3: Above reagents are for a single 96-well microplate

4.1 Required But Not Provided:

- Pipette capable of delivering 0.050ml (50µl) volumes with a precision of better than 1.5%.
- Dispenser(s) for repetitive deliveries of 0.100 and 0.350ml (100 and 350µl) volumes with a precision of better than 1.5%.
- Microplate washers or a squeeze bottle (optional).

- Microplate Reader with 450nm and 620nm wavelength absorbance capability.
- Absorbent Paper for blotting the microplate wells.
- Plastic wrap or microplate cover for incubation steps.
- Vacuum aspirator (optional) for wash steps.
- Timer.
- Quality control materials

5.0 PRECAUTIONS

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

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

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

6.0 SPECIMEN COLLECTION AND PREPARATION

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

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

Samples may be refrigerated at 2-8°C for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20°C for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.100 ml (100 µl) of the specimen is required.

7.0 QUALITY CONTROL

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

8.0 REAGENT PREPARATION

- 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.
- Working Substrate Solution** – Stable for one year
Pour the contents of the amber vial labeled Solution 'A' into the clear vial labeled Solution 'B'. Place the yellow cap on the clear vial for easy identification. Mix and label accordingly. Store at 2 - 8°C.

**Note1: Do not use the working substrate if it looks blue.
Note 2: Do not use reagents that are contaminated or have bacteria growth.**

9.0 TEST PROCEDURE

Before proceeding with the assay, bring all reagents, serum reference calibrators and controls to room temperature (20-27°C).

****Test Procedure should be performed by a skilled individual or trained professional****

- Format the microplate wells for each serum reference calibrator, control and patient specimen to be assayed in duplicate. **Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C**
- Pipette 0.050 ml (50µl) of the appropriate serum reference calibrator, control or specimen into the assigned well.
- Add 0.100 ml (100µl) of LH-Enzyme Reagent to all wells.
- Swirl the microplate gently for 20-30 seconds to mix and cover.
- Incubate 60 minutes at room temperature.
- Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.
- 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.**
- 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
- Incubate at room temperature for fifteen (15) minutes.
- 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**
- Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) in a microplate reader. **The results should be read within thirty (30) minutes of adding the stop solution.**

10.0 CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of luteinizing hormone (LH) in unknown specimens.

- Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.
- Plot the absorbance for each duplicate serum reference versus the corresponding LH concentration in mIU/ml on linear graph paper (do not average the duplicates of the serum references before plotting).
- Draw the best-fit curve through the plotted points.
- To determine the concentration of LH for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in mIU/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance (1.005) intersects the dose response curve at 42.7 mIU/ml LH concentration (See Figure 1).

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

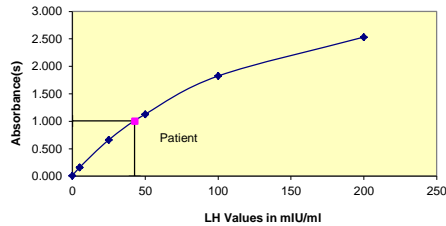
*The data presented in Example 1 and Figure 1 is for illustration only and **should not** be used in lieu of a dose response curve prepared with each assay.

EXAMPLE 1

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

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

Figure 1



11.0 Q.C. PARAMETERS

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

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

12.0 RISK ANALYSIS

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

12.1 Assay Performance

- It is important that the time of reaction in each well is held constant to achieve reproducible results.
- Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
- Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
- If more than one (1) plate is used, it is recommended to repeat the dose response curve.
- 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.
- Plate readers measure vertically. Do not touch the bottom of the wells.
- Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
- Use components from the same lot. No intermixing of reagents from different batches.
- 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.

- 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.
- 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.
- 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

- Measurements and interpretation of results must be performed by a skilled individual or trained professional.**
- 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.
- "The reagents for the test system procedure have been formulated to eliminate maximal interference; however, potential interaction between rare serum specimens and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for all kinds of immunoassays. (Boscato LM Stuart MC. 'Heterophilic antibodies: a problem for all immunoassays' Clin.Chem. 1988:3427-33). For diagnostic purposes, the results from this assay should be used in combination with clinical examination, patient history and all other clinical findings."
- For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
- 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.**
- 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.
- LH is suppressed by estrogen but in woman taking oral contraceptives the level may be low or normal. Excessive dieting and weight loss may lead to low gonadotropin concentrations.
- Luteinizing hormone is dependent upon diverse factors other than pituitary homeostasis. Thus, the determination alone is not sufficient to assess clinical status.

13.0 EXPECTED RANGES OF VALUES

A study of an apparent normal adult population was undertaken to determine expected values for the LH AccuBind® ELISA Test System. The expected values are presented in Table 1.

TABLE 1
Expected Values for the LH ELISA Test System (in mIU/ml)

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

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

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

The within and between assay precisions of the LH AccuBind® ELISA Test System were determined by analyses on three different levels of control sera. The number (N), mean value (X),

standard deviation (σ) and coefficient of variation (C.V.) for each of these control sera are presented in Table 2 and Table 3.

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

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

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

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

*As measured in ten experiments in duplicate.

14.2 Sensitivity

The LH AccuBind® ELISA Test System has a sensitivity of 0.003mIU/well. This is equivalent to a sample containing 0.054 mIU/ml LH concentration. The analytical sensitivity (detection limit) was ascertained by determining the variability of the '0 mIU/ml' calibrator and using the 2σ (95% certainty) statistic to calculate the minimum dose.

14.3 Accuracy

This LH AccuBind® ELISA Test System was compared with a reference radioimmunoassay. Biological specimens from normal, and pregnant populations were assayed. The total number of such specimens was 110. The least square regression equation and the correlation coefficient were computed for the LH ELISA method in comparison with the reference method. The data obtained is displayed in Table 4.

TABLE 4

Method	Mean (x)	Least Square Regression Analysis	Correlation Coefficient
This Method	14.8	$y = 0.081 + 0.93(x)$	0.989
Reference Method	15.1		

Only slight amounts of bias between the LH AccuBind® ELISA Test System and the reference method are indicated by the closeness of the mean values. The least square regression equation and correlation coefficient indicates excellent method agreement.

14.4 Specificity

The cross-reactivity of the LH AccuBind® ELISA Test System to selected substances was evaluated by adding the interfering substance to a serum matrix at various concentrations. The cross-reactivity was calculated by deriving a ratio between dose of interfering substance to dose of Luteinizing Hormone needed to produce the same absorbance.

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

15.0 REFERENCES

- Kosasa T.S., "Measurement of Human Luteinizing Hormone", *Journal of Reproductive Medicine*, 26, 201-6 (1981).
- Danzer H, Braunstein GD, et al, "Maternal Serum Human Chorionic Gonadotropin Concentrations and Fetal Sex Predictions", *Fertility and Sterility*, 34, 336-40 (1980).
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- Goldstein DP, and Kosasa T, "The Subunit Radioimmunoassay for LH Clinical Application", *Gynecology*, 6, 45-84 (1975).
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- Lenton E, Neal L and Sulaiman R, "Plasma Concentrations of Human Gonadotropin from the Time of Implantation until the Second Week of Pregnancy", *Fertility and Sterility*, 37, 773-78 (1982).

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

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

For Orders and Inquires, please contact

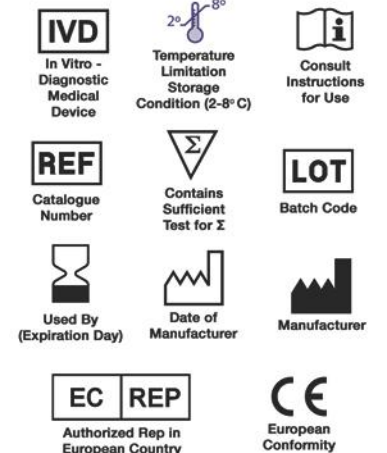


Tel: +1 949.951.2655 Mail: info@monobind.com
Fax: +1 949.951.3539 Fax: www.monobind.com



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

Glossary of Symbols
(EN 980/ISO 15223)





Progesterone Test System Product Code: 4825-300

1.0 INTRODUCTION

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

2.0 SUMMARY AND EXPLANATION OF THE TEST

Measurement of progesterone in serum or plasma is considered to be the most reliable way to assess its rate of production.

Progesterone is a steroid hormone, which plays an important role in the preparation for and maintenance of pregnancy. It is synthesized from cholesterol via pregnenolone — then rapidly metabolized to pregnenediol primarily in the liver.^{2, 9, 13} The ovary and placenta are the major production sites, but a small amount is also produced by the adrenal cortex in both men and women. Circulating progesterone levels, which are characteristically low during the follicular phase, increase sharply during the luteal phase of menstrual cycles, reaching a maximum approximately 5 to 10 days after the midcycle LH peak.¹² Unless pregnancy occurs, a steep decline to follicular levels sets in about 4 days before the next menstrual period. This pattern constitutes the rationale behind the well established use of serum progesterone measurements as a simple and reliable method for ovulation detection.^{3, 4, 16}

For routine measurements, immunoassays using steroid specific antibodies are preferred. Initial immunoassays for serum progesterone used organic solvents to remove the steroid from endogenous binding proteins such as corticosteroid binding globulin (CBG) and albumin. Direct measurement of progesterone in serum or plasma is considered to be the method of choice for routine applications. Both RIA and EIA (and some FIA) are available in the market. Since RIA involves handling radioactivity and causes radioactive waste disposal issues, various non-isotopic methods have replaced the RIA. These methods use very specific antibodies to determine levels of progesterone in circulation.

The Monobind Progesterone ELISA kit uses a specific anti-progesterone antibody, and does not require sample extraction of serum or plasma. Cross-reactivity to other naturally occurring and structurally related steroids is low.

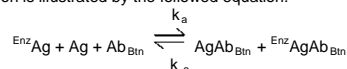
The employment of several serum references of known progesterone concentration permits construction of a graph of activity and concentration. From comparison to the dose response curve, an unknown specimen's activity can be correlated with progesterone concentration.

3.0 PRINCIPLE

Competitive Enzyme Immunoassay (TYPE 7):

The essential reagents required for an enzyme immunoassay include antibody, enzyme-antigen conjugate and native antigen. Upon mixing biotinylated antibody, enzyme-antigen conjugate and a serum containing the native antigen, a competition reaction

results between the native antigen and the enzyme-antigen conjugate for a limited number of antibody binding sites. The interaction is illustrated by the following equation:



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

A simultaneous reaction between the biotin attached to the antibody and the streptavidin immobilized on the microwell occurs. This effects the separation of the antibody bound fraction after decantation or aspiration.

AgAb_{BtN} + Enz AgAb_{BtN} + Streptavidin_{CW} ⇒ immobilized complex
 Streptavidin_{CW} = Streptavidin immobilized on well
immobilized complex = sandwich complex bound to the solid surface

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

4.0 REAGENTS

Materials Provided:

A. Progesterone Calibrators – 1ml/vial - Icons A-G

Seven (7) vials of serum reference for progesterone at concentrations of 0 (A), 0.3 (B), 2.0 (C), 5.0 (D), 15 (E), 30 (F) and 60.0 (G) ng/ml. Store at 2-8°C. A preservative has been added. The calibrators can be expressed in molar concentrations (nM/L) by multiplying by 3.18. For example: 1ng/ml x 3.18 = 3.18 nM/L

B. Progesterone Enzyme Reagent – 6ml/vial – Icon

One (1) vial of Progesterone (Analog)-horseradish peroxidase (HRP) conjugate in a protein stabilizing matrix with red dye. Store at 2-8°C.

C. Progesterone Biotin Reagent – 6ml/vial - Icon

One (1) vial of reagent contains anti-Progesterone biotinylated purified rabbit IgG conjugate in buffer, yellow dye and preservative. Store at 2-8°C.

D. Streptavidin Coated Plate – 96 wells –Icon

One 96-well microplate coated with 1.0 µg/ml streptavidin and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

E. Wash Solution Concentrate – 20ml/vial – Icon

One (1) vial contains a surfactant in buffered saline. A preservative has been added. Store at 2-8°C.

F. Substrate Reagent – 12ml/vial - Icon

One (1) vial contains tetramethylbenzidine (TMB) and hydrogen peroxide (H₂O₂) in buffer. Store at 2-8°C.

G. Stop Solution – 8ml/vial - Icon

One (1) vial contains a strong acid (H₂SO₄). 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:

- Pipette capable of delivering 0.025 & 0.050ml (25 & 50µl) with a precision of better than 1.5%.
- Dispenser(s) for repetitive deliveries of 0.100 & 0.350ml (100 & 350µl) volumes with a precision of better than 1.5%.
- Adjustable volume (200-1000µl) dispenser(s) for conjugate.
- Microplate washer or a squeeze bottle (optional).
- Microplate Reader with 450nm and 620nm wavelength absorbance capability.
- Absorbent Paper for blotting the microplate wells.
- Plastic wrap or microplate cover for incubation steps.
- Vacuum aspirator (optional) for wash steps.
- Timer.

10. Quality control materials.

5.0 PRECAUTIONS

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

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

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

6.0 SPECIMEN COLLECTION AND PREPARATION

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

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

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

7.0 QUALITY CONTROL

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

8.0 REAGENT PREPARATION

1. Wash Buffer

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

Note: Do not use reagents that are contaminated or have bacteria growth.

9.0 TEST PROCEDURE

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

- 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.**

- Pipette 0.025ml (25µl) of the appropriate serum reference calibrator, control or specimen into the assigned well.
- Add 0.050ml (50µl) of Progesterone Enzyme Reagent to all wells.
- Swirl the microplate gently for 10-20 seconds to mix.
- Add 0.050ml (50µl) Progesterone Biotin Reagent to all wells.
- Swirl the microplate gently for 10-20 seconds to mix.
- Cover and incubate for 60 minutes at room temperature.
- Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.
- 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.**
- Add 0.100ml (100µl) of Substrate reagent to all wells. **Always add reagents in the same order to minimize reaction time differences between wells.**

DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION

- Incubate at room temperature for twenty (20) minutes.
- 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.**
- Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm). **The results should be read within fifteen (15) minutes of adding the stop solution.**

Note: Dilute the samples suspected of concentrations higher than 60ng/ml 1:5 and 1:10 with progesterone '0' ng/ml calibrator or male patient serum pools with a known low value for progesterone.

10.0 CALCULATION OF RESULTS

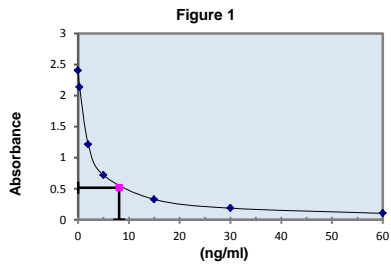
A dose response curve is used to ascertain the concentration of progesterone in unknown specimens.

- Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.
- Plot the absorbance for each duplicate serum reference versus the corresponding progesterone concentration in ng/ml on linear graph paper.
- Connect the points with a best-fit curve.
- To determine the concentration of progesterone 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 (0.517) intersects the dose response curve at 8.1ng/ml progesterone concentration.

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

EXAMPLE 1

Sample I.D.	Well Number	Abs (A)	Mean Abs (B)	Value (ng/ml)
Cal A	A1	2.420	2.406	0
	B1	2.391		
Cal B	C1	2.155	2.137	0.3
	D1	2.119		
Cal C	E1	1.248	1.215	2.0
	F1	1.183		
Cal D	G1	0.721	0.719	5.0
	H1	0.717		
Cal E	A2	0.338	0.330	15.0
	B2	0.322		
Cal F	C2	0.187	0.188	30.0
	D2	0.190		
Cal G	G2	0.107	0.105	60.0
	H2	0.104		
Pat# 1	A3	0.525	0.517	8.1
	B3	0.510		



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

11.0 Q.C. PARAMETERS

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

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

12.0 RISK ANALYSIS

The MSDS and Risk Analysis Form for this product are available upon 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. Patient specimens with Progesterone levels higher than 60ng/ml may be diluted (1:5 or 1:10) with progesterone '0 ng/ml' calibrator or male patient serum pools with a known low value for progesterone.
11. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.
12. It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.
13. Risk Analysis- as required by CE Mark IVD Directive 98/79/EC - for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com.

12.2 Interpretation

1. **Measurements and interpretation of results must be performed by a skilled individual or trained professional.**
2. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.
3. The reagents for the test system procedures 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, results from this assay should be used in combination with clinical examination, patient history and all other clinical findings.

4. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
5. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, **Monobind shall have no liability.**
6. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.

13.0 EXPECTED RANGES OF VALUES

In agreement with established reference intervals for a "normal" adult population and females during gestation the expected ranges for the Progesterone AccuBind® ELISA Test System are detailed in Table 1. During pregnancy the progesterone serum levels rise rapidly till the end of third trimester.¹⁷

TABLE 1
Expected Values for the Progesterone Test System

	(ng/ml)	(nmol/L)
Prepubertal Child (1-10 yr)	0.07 – 0.52	0.2-1.7
Adult man	0.13 – 1.22	0.4 – 3.88
Adult woman		
Follicular phase	0.15 – 1.40	0.5 – 4.4
Luteal phase	2.0 – 25.0	6.4 – 79.5
Pregnant woman		
First trimester	7.25 – 90.0	23 – 286
Second trimester	19.5 – 91.0	62 – 289
Third trimester	49.0 – 422.0	153 – 1342
Postmenopausal woman	0.0 – 0.80	0.0 – 2.55

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 until an in-house range can be determined by 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 Progesterone AccuBind® ELISA Test System were determined by analyses on three different levels of pool control sera. The number, mean values, standard deviation and coefficient of variation for each of these control sera are presented in Table 2 and Table 3.

TABLE 2

Within Assay Precision (Values in ng/ml)				
Sample	N	X	σ	C.V.%
Low	20	0.65	0.100	15.3
Normal	20	10.77	0.405	3.8
High	20	24.94	1.528	6.1

TABLE 3

Between Assay Precision (Values in ng/ml)				
Sample	N	X	σ	C.V.%
Low	20	0.72	0.065	8.9
Normal	20	10.88	0.846	7.5
High	20	24.05	1.534	6.4

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

14.2 Sensitivity

The Progesterone AccuBind® ELISA Test System has a sensitivity of 0.105 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 Progesterone AccuBind® ELISA Test System was compared with a chemiluminescence immunoassay method. Biological specimens from low, normal and high progesterone level populations were used (values ranged from < 0.15 ng/ml – 128 ng/ml). The total number of such specimens was 60. The least square regression equation and the correlation coefficient were computed for this 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)	14.59	$y = -1.223 + 1.018(x)$	0.989
Reference (X)	15.53		

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 progesterone 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 progesterone needed to displace the same amount of labeled analog.

Substance	Cross Reactivity
Progesterone	100.000
17OH-Progesterone	0.375
Androstenedione	0.158
Cortisone	0.014
Corticosterone	0.347
Cortisol	0.005
Danazol	0.003
Dihydrotestosterone	0.006
DHEA sulfate	0.002
Estradiol	0.004
Estrone	0.003
Estril	0.002
Prednisone	0.023
Testosterone	0.015

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Revision: 7 Date: 2019-Jul-16 DCO: 1353
MP4825 Product Code: 4825-300

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

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100 North Pointe Drive
Lake Forest, CA 92630 USA

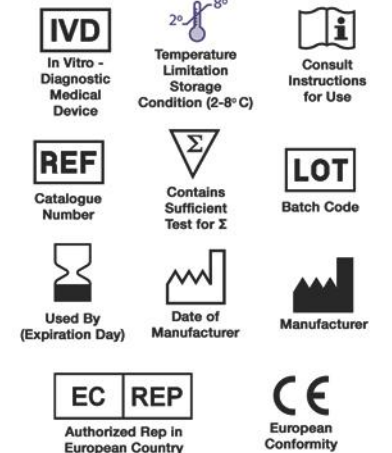
Tel: +1 949.951.2665 Mail: info@monobind.com
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Glossary of Symbols

(EN 980/ISO 15223)





Prolactin Hormone (PRL) Sequential Test System
Product Code: 4425-300

1.0 INTRODUCTION

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

2.0 SUMMARY AND EXPLANATION OF THE TEST

Prolactin hormone (PRL), secreted from the lactotrophs of the anterior pituitary, is a protein consisting of a single polypeptide chain containing approximately 200 amino acids. The primary biological action of the hormone is on the mammary gland where it is involved in the growth of the gland and in the induction and maintenance of milk production. There is evidence to suggest that prolactin may be involved in steroidogenesis in the gonad, acting synergistically with luteinizing hormone (LH). High levels of prolactin appear to inhibit steroidogenesis as well as inhibiting LH and follicle stimulating hormone (FSH) synthesis at the pituitary gland.^{1,2}

The clinical usefulness of the measurement of prolactin hormone (PRL) in ascertaining the diagnosis of hyperprolactinemia and for the subsequent monitoring the effectiveness of the treatment has been well established.^{3,4}

In this method, PRL calibrator, patient specimen or control is first added to a streptavidin coated well. Biotinylated monoclonal antibody (specific for PRL) is added and the reactants mixed. Reaction between the PRL antibodies and native PRL forms complex that binds with the streptavidin coated to the well. The excess serum proteins are washed away via a wash step. An enzyme labeled monoclonal antibody specific to PRL (different epitope from the biotinylated antibody) is added to the wells. The enzyme labeled antibody binds to the PRL 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 PRL in the sample.

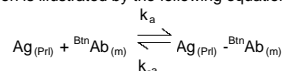
3.0 PRINCIPLE

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-prolactin antibody.

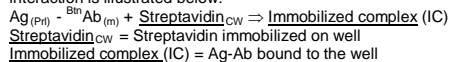
Upon mixing monoclonal biotinylated antibody, and a serum containing the native antigen, reaction results between the native

antigen and the antibody, forming an antibody-antigen complex. The interaction is illustrated by the following equation:

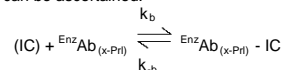


$B^{in}Ab_{(m)}$ = Biotinylated Monoclonal Antibody (Excess Quantity)
 $Ag_{(Prl)}$ = Native Antigen (Variable Quantity)
 $Ag_{(Prl)} - B^{in}Ab_{(m)}$ = Antigen-Antibody complex (Variable Quantity)
 k_a = Rate Constant of Association
 k_{-a} = Rate Constant of Disassociation

Simultaneously, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antibody. This interaction is illustrated below:



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.



$EnzAb_{(x-Prl)}$ = Enzyme labeled Antibody (Excess Quantity)
 $EnzAb_{(x-Prl)} - IC$ = Antigen-Antibodies Complex
 k_b = Rate Constant of Association
 k_{-b} = Rate Constant of Disassociation

4.0 REAGENTS

Materials Provided:

- A. PRL-seq Calibrators – 1 ml/vial - Icons A-F**
Six (6) vials of references for PRL antigen in human serum at levels of 0(A), 10(B), 25(C), 50(D), 100(E) and 250(F) ng/ml*. Store at 2-8°C. A preservative has been added.
***Note:** The calibrators, human serum based, were calibrated using a reference preparation, which was assayed against the WHO 3rd, IS (84/500).
- B. PRLs Biotin Reagent – 13ml/vial - Icon V**
One (1) vial containing biotinylated monoclonal mouse IgG in buffer, dye, and preservative. Store at 2-8°C.
- C. PRLs Enzyme Reagent – 13ml/vial - Icon E**
One (1) vial containing enzyme (HRP) labeled antibody, in buffer, dye, and preservative. Store at 2-8°C.
- D. Streptavidin Coated Microplate – 96 wells - Icon J**
One 96-well microplate coated with streptavidin and packaged in an aluminum bag with a drying agent. Store at 2-8°C.
- E. Wash Solution Concentrate – 20 ml - Icon D**
One (1) vial containing a surfactant in buffered saline. A preservative has been added. Store at 2-8°C.
- F. Substrate A – 7ml/vial - Icon S^A**
One (1) vial containing tetramethylbenzidine (TMB) in buffer. Store at 2-8°C.
- G. Substrate B – 7ml/vial - Icon S^B**
One (1) vial containing hydrogen peroxide (H₂O₂) in buffer. Store at 2-8°C.
- H. Stop Solution – 8ml/vial - Icon S^{OP}**
One (1) vial containing a strong acid (1N HCl). Store at 2-8°C.
- I. Product Instructions.**

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

4.1 Required But Not Provided:

1. Pipette capable of delivering 0.025ml (25µl) and 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 washers or a squeeze bottle (optional).
4. Microplate reader with 450 & 620nm filters.
5. Absorbent Paper for blotting the microplate wells.
6. Plastic wrap or microplate cover for incubation steps.
7. Vacuum aspirator (optional) for wash steps.
8. Timer.
9. Quality control materials

5.0 PRECAUTIONS

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

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

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

6.0 SPECIMEN COLLECTION AND PREPARATION

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

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

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

7.0 QUALITY CONTROL

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

8.0 REAGENT PREPARATION

1. **Wash Buffer**
Dilute contents of wash 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 year
Pour the contents of the amber vial labeled Solution 'A' into the clear vial labeled Solution 'B'. Place the yellow cap on the clear vial for easy identification. Mix and label accordingly. Store at 2 - 8°C.

- Note1:** Do not use the working substrate if it looks blue.
- Note 2:** Do not use reagents that are contaminated or have bacteria growth.

9.0 TEST PROCEDURE

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

1. Format the microplate wells for each serum reference calibrator, control and patient specimen to be assayed in duplicate. **Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C**
2. Pipette 0.025 ml (25µl) of the appropriate serum reference calibrator, control or specimen into the assigned well.
3. Add 0.100 ml (100µl) of Prolactin Biotin Reagent to all wells.
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, 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 Prolactin Enzyme Reagent solution to all wells. **DO NOT SHAKE THE PLATE AFTER ENZYME ADDITION**
9. Incubate 30 minutes at room temperature.
10. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.
11. Add 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 differences between wells. DO NOT SHAKE 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). **Always add reagents in the same order to minimize reaction time differences between wells**
15. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm 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 prolactin hormone (PRL) in unknown specimens.

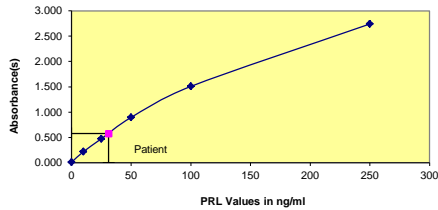
1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the absorbance for each duplicate serum reference versus the corresponding PRL concentration in ng/ml on linear graph paper (do not average the duplicates of the serum references before plotting).
3. Draw the best-fit curve through the plotted points.
4. To determine the concentration of PRL 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 (0.576) intersects the dose response curve at (31.1 ng/ml) PRL concentration (See Figure 1).

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

EXAMPLE 1

Sample I.D.	Well Number	Abs (A)	Mean Abs (B)	Value (ng/ml)
Cal A	A1	0.012	0.010	0
	B1	0.007		
Cal B	C1	0.235	0.219	10
	D1	0.202		
Cal C	E1	0.482	0.474	25
	F1	0.465		
Cal D	G1	0.883	0.896	50
	H1	0.910		
Cal E	A2	1.537	1.508	100
	B2	1.480		
Cal F	C2	2.760	2.714	250
	D2	2.669		
Patient	G2	0.610	0.576	31.1
	H2	0.543		

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 QC. PARAMETERS

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

- The absorbance (OD) of calibrator F should be ≥ 1.3
- 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

- It is important that the time of reaction in each well is held constant to achieve reproducible results.
- Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
- Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
- If more than one (1) plate is used, it is recommended to repeat the dose response curve.
- 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.
- Plate readers measure vertically. Do not touch the bottom of the wells.
- Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
- Use components from the same lot. No intermixing of reagents from different batches.
- 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.
- 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.
- 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.

- Risk Analysis- as required by CE Mark IVD Directive 98/79/EC - for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com.

12.2 Interpretation

1. **Measurements and interpretation of results must be performed by a skilled individual or trained professional.**

2. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.

3. The reagents for the test system have been formulated to eliminate maximal interference; however, potential interaction between rare serum specimens and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for all kinds of immunoassays (Boscato LM, Stuart MC. 'Heterophilic antibodies: a problem for all immunoassays' Clin. Chem. 1988:3427-33). For diagnostic purposes, the results from this assay should be in combination with clinical examination, patient history and all other clinical findings.

4. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.

5. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, **Monobind shall have no liability.**

6. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.

7. Patient specimens with abnormally high prolactin levels will not cause a hook effect, due to the assay design (sequential method). For specimens with values greater than 250, dilute the specimen 1/50 with '0' calibrator, and re-assay (multiply the result by 50).

8. Patients receiving preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA) and may show either falsely elevated or depressed values when assayed.

9. Pregnancy, lactation, and the administration of oral contraceptives can cause an increase in the level of Prolactin.

10. Drugs such as morphine, reserpine and the psychotropic drugs increase prolactin secretion.^{5,6,7}

11. Since Prolactin hormone concentration is dependent upon diverse factors other than pituitary homeostasis, the determination alone is not sufficient to assess clinical status.

13.0 EXPECTED RANGES OF VALUES

A study of an apparent normal adult population was undertaken to determine expected values for the PRL-seq AccuBind® ELISA Test System. The expected values (95% confidence intervals) are presented in Table 1.

TABLE 1
Expected Values for the PRL-seq AccuBind® ELISA (in ng/ml)

Women	
Adult (Number = 70)	1.2 -- 19.5
Postmenopausal (Number = 10)	1.5 -- 18.5
Men	
Adult (Number = 50)	1.8 -- 17.0

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

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

The within and between assay precision of the PRL-seq AccuBind® ELISA test system were determined by analyses on three different levels of control sera. The number, mean value, standard deviation (σ) and coefficient of variation for each of these control sera are presented in Table 3 and Table 4.

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

Sample	N	X	σ	C.V.
Level 1	24	10.6	0.35	3.3%
Level 2	24	28.6	0.84	3.0%
Level 3	24	77.5	1.93	2.5%

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

Sample	N	X	σ	C.V.
Level 1	10	11.5	0.19	1.7%
Level 2	10	27.8	0.50	1.8%
Level 3	10	78.5	2.32	3.0%

*As measured in ten experiments in duplicate.

14.2 Sensitivity

This procedure has a sensitivity of 0.04 ng. This is equivalent to a sample containing 0.8 ng/ml PRL concentration. The sensitivity was ascertained by determining the variability of the '0 ng/ml' calibrator and using the 2σ (95% certainty) statistic to calculate the minimum dose.

14.3 Accuracy

The PRL-seq AccuBind® ELISA test system was compared with a reference method. Biological specimens from normal and pregnant populations were assayed. The total number of such specimens was 86. The least square regression equation and the correlation coefficient were computed for the PRL ELISA in comparison with the reference method. The data obtained is displayed in Table 4.

Method	TABLE 4		
	Mean (x)	Least Square Regression Analysis	Correlation Coefficient
Monobind Reference	19.0	$y = 1.63 + 1.01(x)$	0.973

Only slight amounts of bias between the PRL-seq 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 prolactin hormone method 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 prolactin hormone needed to produce the same absorbance.

Substance	Cross Reactivity	Concentration
Prolactin Hormone (PRL)	1.0000	--
Luteinizing Hormone (LH)	< 0.0001	1000ng/ml
Follitropin (FSH)	< 0.0001	1000ng/ml
Chorionic gonadotropin (CG)	< 0.0001	1000ng/ml
Thyrotropin (TSH)	< 0.0001	1000ng/ml
Growth Hormone (GH)	< 0.0001	1000ng/ml

15.0 REFERENCES

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Revision: 4 Date: 2019-Jul-16 DCO: 1353
MP4425 Product Code: 4425-300

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

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Glossary of Symbols
(EN 980/ISO 15223)



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.

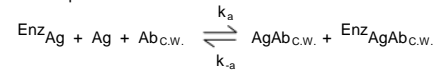
After the completion of the required incubation period, the antibody bound enzyme-thyroxine conjugate is separated from the unbound enzyme-thyroxine conjugate via a wash step. The activity of the enzyme present on the surface of the well is quantitated by reaction with a suitable substrate to produce 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 following equation:



- 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_{AgAb_{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 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 Y**
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 D**
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 (H₂O₂) in acetate buffer. Store at 2-8°C.
- G. Stop Solution – 8 ml/vial - Icon S^{TOP}**
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°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 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.

- 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 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**

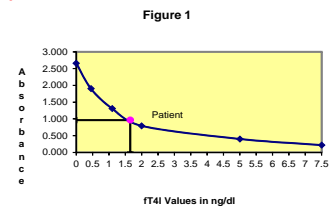
- 2. Pipette 0.050 ml (50µl) of the appropriate serum reference, control or specimen into the assigned well.
- 3. Add 0.100 ml (100µl) of FT4 Enzyme Reagent to all wells.
- 4. Swirl the microplate gently for 20-30 seconds to mix and cover.
- 5. Incubate 60 minutes at room temperature.
- 6. Discard the 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.**



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:

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

12.0 RISK ANALYSIS

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

12.1 Assay Performance

- It is important that the time of reaction in each well is held constant to achieve reproducible results.
 - Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
 - Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
 - If more than one (1) plate is used, it is recommended to repeat the dose response curve.
 - 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.
 - Plate readers measure vertically. Do not touch the bottom of the wells.
 - Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
 - Use components from the same lot. No intermixing of reagents from different batches.
 - 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.
 - 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.
 - 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.
 - 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
- Measurements and interpretation of results must be performed by a skilled individual or trained professional.
 - 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.
 - For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
 - 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.**
 - 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.
 - 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.

- 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.
- Serum free-thyroxine values may be elevated under conditions such as pregnancy or administration of oral contraceptives.
- 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.
- 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.
- 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.
- Circulating antibodies to T4 and hormone binding inhibitors may interfere in the performance of the assay.
- 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 ($\pm 2\sigma$) are presented in Table 1.

	Adult	Pregnancy
Number of Specimens	89	31
Mean (X)	1.40	1.50
Standard Deviation (σ)	0.30	0.37
Expected Ranges ($\pm 2\sigma$)	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.

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.

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).

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 n
I-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
TBG	N/D	40 µg/ml
Albumin	N/D	40 mg/ml
Phenylbutazone	N/D	10 µg/ml
Phenytol	N/D	40 µg/ml
Salicylates	N/D	500 µg/ml

15.0 REFERENCES

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

Size	96(A)	192(B)	480(D)	960(E)
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)

For Orders and Inquiries, please contact

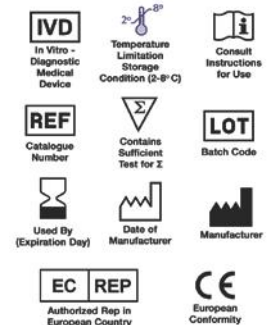
Monobind Inc.
100 North Pointe Drive
Lake Forest, CA 92650 USA

Tel: +1 949.951.2665 Mail: info@monobind.com
Fax: +1 949.951.3539 Fax: www.monobind.com



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

Glossary of Symbols
(EN 95000 15222)





Testosterone Test System Product Code: 3725-300

1.0 INTRODUCTION

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

2.0 SUMMARY AND EXPLANATION OF THE TEST

Testosterone, (17 β -Hydroxy-4-androstene-3-one), a C₁₉ steroid, is the most potent naturally secreted androgen.¹ In normal post pubertal males, testosterone is secreted primarily by the testes with only a small amount derived from peripheral conversion of 4-Androstene-3, 17-dione (ASD).² In adult women, it has been estimated that over 50% of serum testosterone is derived from peripheral conversion of ASD secreted by the adrenal and ovary, with the remainder from direct secretion of testosterone by these glands.

In the male, testosterone is mainly synthesized in the interstitial Leydig cells and the testis, and is regulated by the interstitial cell stimulating hormone (ICSH), or luteinizing hormone (LH) of the anterior pituitary (the female equivalent of ICSH).³ Testosterone is responsible for the development of secondary sex characteristics, such as the accessory sex organs, the prostate, seminal vesicles and the growth of facial, pubic and auxiliary hair. Testosterone measurements have been very helpful in evaluating hypogonadal states. Increased testosterone levels in males can be found in complete androgen resistance (testicular feminization). Common causes of decreased testosterone levels in males include: hypogonadism, orchidectomy, estrogen therapy, Klinefelter's syndrome, hypopituitarism, and hepatic cirrhosis.^{2,4}

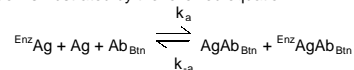
In the female, testosterone levels are normally found to be much lower than those encountered in the healthy male. Testosterone in the female comes from three sources. It is secreted in small quantities by both the adrenal glands and the ovaries, and in healthy women 50-60% of the daily testosterone production arises from peripheral metabolism of prohormone, chiefly androstenedione. Common causes of increased serum testosterone levels in females include polycystic ovaries (Stein-Leventhal syndrome), ovarian tumors, adrenal tumors and adrenal hyperplasia. Virilization in women is associated with the administration of androgens and endogenous overproduction of testosterone. There appears to be a correlation between serum testosterone levels and the degree of virilization in women, although approximately 25% of women with varying degrees of virilism have serum testosterone levels that fall within the female reference range.

3.0 PRINCIPLE

Competitive Enzyme Immunoassay (TYPE 7):

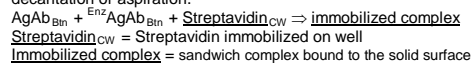
The essential reagents required for an enzyme immunoassay include antibody, enzyme-antigen conjugate and native antigen.

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



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

A simultaneous reaction between the biotin attached to the antibody and the streptavidin immobilized on the microwell occurs. This effects the separation of the antibody bound fraction after decantation or aspiration.



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. Testosterone Calibrators – 1ml/vial – Icons A-G

Seven (7) vials of serum reference for Testosterone at concentrations of 0 (A), 0.1 (B), 0.5 (C), 1.0 (D), 2.5 (E), 5.0 (F) and 12.0 (G) in ng/ml. Store at 2-8°C. A preservative has been added. The calibrators can be expressed in molar concentrations (nM/L) by multiplying by 3.47. For example: 1ng/ml x 3.47 = 3.47 nM/L

B. Testosterone Enzyme Reagent – 6.0 ml/vial – Icon E

One (1) ready to use vial of Testosterone (Analog)-horse radish peroxidase (HRP) conjugate in a protein stabilizing matrix with buffer, red dye, preservative, and binding protein inhibitors. Store 2-8°C.

C. Testosterone Biotin Reagent – 6.0 ml – Icon V

One (1) vial containing anti-Testosterone biotinylated purified rabbit IgG conjugate in buffer, dye and preservative. Store at 2-8°C.

D. Streptavidin Coated Plate – 96 wells – Icon J

One 96-well microplate coated with 1.0 µg/ml streptavidin and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

E. Wash Solution Concentrate – 20ml/vial – Icon L

One (1) vial containing a surfactant in buffered saline. A preservative has been added. Store at 2-8°C.

F. Substrate A – 7ml/vial – Icon S^A

One (1) vial containing tetramethylbenzidine (TMB) in buffer. Store at 2-8°C.

G. Substrate B – 7ml/vial – Icon S^B

One (1) vial containing hydrogen peroxide (H₂O₂) in buffer. Store at 2-8°C.

H. Stop Solution – 8ml/vial – Icon STOP

One (1) vial containing a strong acid (1N HCl). Store at 2-8°C.

I. Product Instructions.

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

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

Note 3: Above reagents are for a single 96-well microplate.

4.1 Required But Not Provided:

- Pipette capable of delivering 0.010ml (10µl), 0.050ml(50µl), 0.100ml (100µl) volumes with a precision of better than 1.5%.

- Dispenser(s) for repetitive deliveries of 0.50ml (50µl) .0.100ml (100 µl) and 0.350ml (350µl) volumes with a precision of better than 1.5%.
- Microplate washer or a squeeze bottle (optional).
- Microplate Reader with 450nm and 620nm wavelength absorbance capability.
- Absorbent Paper for blotting the microplate wells.
- Plastic wrap or microplate covers for incubation steps.
- Vacuum aspirator (optional) for wash steps.
- Timer.
- Quality control materials.

5.0 PRECAUTIONS

For In Vitro Diagnostic Use

Not for Internal or External Use in Humans or Animals

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

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

6.0 SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood; serum or plasma in type and the usual precautions in the collection of venipuncture samples should be observed. For accurate comparison to established normal values, a fasting morning serum sample should be obtained. The blood should be collected in a plain redtop venipuncture tube or (for plasma) in evacuated tube(s) containing heparin. Allow the blood to clot for serum samples. Centrifuge the specimen to separate the serum or plasma from the cells.

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

Samples may be refrigerated at 2-8°C for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20°C for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.020ml (20µl) of the specimen is required.

7.0 QUALITY CONTROL

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

8.0 REAGENT PREPARATION

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

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 calibrators and controls to room temperature (20 - 27°C).

****Test Procedure should be performed by a skilled individual or trained professional****

- Format the microplates' wells for each serum reference, control and patient specimen to be assayed in duplicate. **Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.**
- Pipette 0.010 ml (10µL) of the appropriate serum reference, control or specimen into the assigned well.
- Add 0.050 ml (50µl) of the ready to use Testosterone Enzyme Reagent to all wells.
- Swirl the microplate gently for 20-30 seconds to mix.
- Add 0.050 ml (50µl) of Testosterone Biotin Reagent to all wells.
- Swirl the microplate gently for 20-30 seconds to mix.
- Cover and incubate for 60 minutes at room temperature.
- Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.
- 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.**
- 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
- Incubate at room temperature for fifteen (15) minutes.
- 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.**
- Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) in a microplate reader. **The results should be read within thirty (30) minutes of adding the stop solution.**

Note: Dilute the samples suspected of concentrations higher than 12 ng/ml 1:5 and 1:10 with Testosterone 'O' ng/ml calibrator or female patient sera with a known low value for testosterone.

10.0 CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of Testosterone in unknown specimens.

- Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.
- Plot the absorbance for each duplicate serum reference versus the corresponding Testosterone concentration in ng/ml on linear graph paper (do not average the duplicates of the serum references before plotting).
- Connect the points with a best-fit curve.
- To determine the concentration of Testosterone for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in ng/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance (1.764) intersects the dose response curve at (0.57ng/ml) Testosterone concentration (See Figure 1).

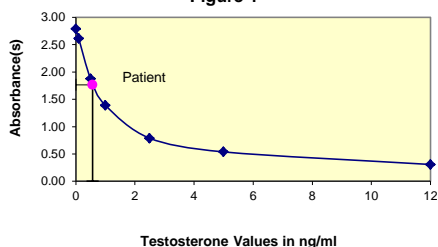
Note: Computer data reduction software designed for ELISA assays may also be used for the data reduction. **If such software is utilized, the validation of the software should be ascertained.**

EXAMPLE 1

Sample I.D.	Well Number	Abs (A)	Mean Abs (B)	Value (ng/ml)
Cal A	A1	2.780	2.787	0
	B1	2.794		
Cal B	C1	2.576	2.611	0.1
	D1	2.646		
Cal C	E1	1.789	1.877	0.5
	F1	1.965		
Cal D	G1	1.391	1.392	1.0
	H1	1.393		
Cal E	A2	0.780	0.788	2.5
	B2	0.796		
Cal F	C2	0.530	0.538	5.0
	D2	0.547		
Cal G	E2	0.301	0.308	12.0
	F2	0.314		
Ctrl 1	G2	1.040	0.760	1.61
	H2	1.045		
Patient	A3	1.751	1.764	0.57
	B3	1.778		

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

Figure 1



11.0 Q.C. PARAMETERS

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

- The absorbance (OD) of calibrator 0 ng/ml should be ≥ 1.3
- 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

- It is important that the time of reaction in each well is held constant to achieve reproducible results.
- Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
- Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
- If more than one (1) plate is used, it is recommended to repeat the dose response curve.
- 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.
- Plate readers measure vertically. Do not touch the bottom of the wells.
- Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
- Use components from the same lot. No intermixing of reagents from different batches.
- 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.

- 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.
- 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

- Measurements and interpretation of results must be performed by a skilled individual or trained professional.** 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.
- The reagents for the procedure have been formulated to eliminate maximal interference; however, potential interaction between rare serum specimens and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for all kinds of immunoassays. (*Boscato LM, Stuart MC, 'Heterophilic antibodies: a problem for all immunoassays' Clin. Chem 1988:3427-33*). For diagnostic purposes the results from this assay should be used in combination with clinical examination, patient's history and, all other clinical findings.
- For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
- 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.**
- If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.

13.0 EXPECTED RANGES OF VALUES

In agreement with established reference intervals⁵ for a "normal" adult population, the expected ranges for the Testosterone AccuBind® ELISA Test System are detailed in Table 1.

TABLE 1
Expected Values for Testosterone EIA Test System (ng/ml)

Boys Before Puberty	0.1 – 3.7
Male	2.5 – 10.0
Female	0.2 – 0.95

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

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

Sample	N	X	σ	C.V.
Low	22	1.63	0.16	9.8%
Normal	22	9.14	0.44	4.8%
High	22	14.22	0.79	5.6%

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

Sample	N	X	σ	C.V.
Low	24	1.72	0.16	9.1%
Normal	24	7.06	0.69	9.7%
High	24	13.08	1.03	7.9%

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

14.2 Sensitivity

The Testosterone AccuBind® ELISA Test System has a sensitivity of 0.576 pg. This is equivalent to a sample containing a concentration of 0.0576 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 Testosterone AccuBind® ELISA Test System was compared with a chemiluminescence immunoassay method. Biological specimens from low, normal and high Testosterone level populations were used. The values ranged from 0.29 ng/ml – 21.9ng/ml. The total number of such specimens was 58. The least square regression equation and the correlation coefficient were computed for this Testosterone EIA in comparison with the reference method. The data obtained is displayed in Table 4.

TABLE 4

Method	Mean (x)	Least Square Regression Analysis	Correlation Coefficient
Monobind (y)	3.12	$y = -0.265 + 0.944(x)$	0.985
Reference (X)	3.02		

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 testosterone 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 Testosterone needed to displace the same amount of labeled analog.

Substance	Cross Reactivity
Testosterone	1.0000
Androstenedione	0.0009
Dihydrotestosterone	0.0178
Cortisone	<0.0001
Corticosterone	<0.0001
Cortisol	<0.0001
Spirolactone	<0.0001
Progesterone	<0.0001
17 α -OH Progesterone	<0.0001
DHEA sulfate	<0.0001
Estradiol	<0.0001
Estrone	<0.0001
Estriol	<0.0001
Hemolysis	<0.0001
Rubella	<0.0001
Lipemia	<0.0001

15.0 REFERENCES

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- Horton R, Tait JF: Androstenedione production and interconversion rates measured in peripheral blood and studies on the possible site of conversion to testosterone. J.Clin Invest 45: 301-303, 1966.
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- Sizonenka, PC, *Pediatrician*, 14, 191 (1987).
- Cummings DC, Wall SR: Non sex hormone binding globulin bound testosterone as a marker for hyperandrogenism. J. Clin Endocrinol Metab. 61:873-876, 1985.
- Lashansky, G, et. al., *J Clin Endocrinol Metab*, 58, 674 (1991)
- Tietz, NW, ED: Clinical Guide to Laboratory Tests, 3rd ed. Philadelphia, WA Saunders Co, 1995.

Size	96(A)	192(B)
Reagent (fill)	A) 1ml set	1ml set
	B) 1 (6ml)	2 (6ml)
	C) 1 (6ml)	2 (6ml)
	D) 1 plate	2 plates
	E) 1 (20ml)	1 (20ml)
	F) 1 (7ml)	2 (7ml)
	G) 1 (7ml)	2 (7ml)
	H) 1 (8ml)	2 (8ml)

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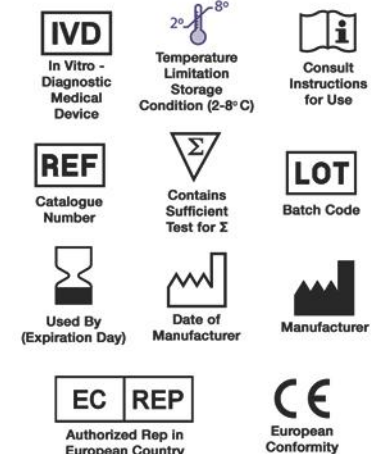


Tel: +1 949.951.2665 Mail: info@monobind.com
Fax: +1 949.951.3539 Fax: www.monobind.com



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Glossary of Symbols
(EN 980/ISO 15223)





Thyroglobulin (Tg) Test System Product Code: 2225-300

1.0 INTRODUCTION

Intended Use: The Quantitative Determination of Thyroglobulin (Tg) Concentration in Human Serum by a Microplate Enzyme immunoassay, Colorimetric

2.0 SUMMARY AND EXPLANATION OF THE TEST

Human thyroglobulin (Tg) is a large glycoprotein (660 kD) that is stored in the follicular colloid of the thyroid gland. It functions as a prohormone in the intrathyroid synthesis of primary thyroid hormones like *Triiodothyronine (T3)* and *Thyroxine (T4)*.

Tg is elevated in thyroid follicular and papillary carcinoma, thyroid adenoma, subacute thyroiditis, Hashimoto's thyroiditis and Graves Disease. Tg levels are found to be normal in patients with medullary thyroid carcinoma. Serial measurements of Tg is most useful in detecting recurrence of differentiated thyroid carcinoma following surgical resection or radioactive iodine ablation. Tg determination is used as an adjunct to iodine scanning but not as a replacement for it. Assessment of Tg levels aids in management of infants with congenital hypothyroidism.

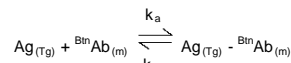
Tg determination has been done with various methods using direct competitive binding RIA and double antibody sandwich IRMA or ELISA, of which latter is more useful. All these methods suffer from interference by endogenous autoantibodies to Tg. It is useful to determine the effect of autoantibodies before screening such patients for levels of Tg. *Monobind* provides Tg autoantibody ELISA to rule out such interference. (**Please see *Monobind Anti-Tg AccuBind® ELISA Test System, Product Code: 1025-300***).

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 Thyroglobulin antibody.

When monoclonal biotinylated antibody is mixed with a serum containing the Tg antigen, a reaction results between the Tg antigen and the antibody, to form an antibody-antigen complex. Simultaneously the biotin attached to the antibody binds to the streptavidin coated on the microwells resulting in immobilization of the complex. The interaction is illustrated by the following equation:

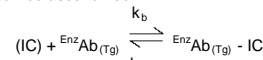


$B^{in}Ab_{(m)}$ = Biotinylated Monoclonal Antibody (Excess Quantity)
 $Ag_{(Tg)}$ = Native Antigen (Variable Quantity)
 $Ag_{(Tg)} - B^{in}Ab_{(m)}$ = Antigen-Antibody complex (Variable Quan.)

k_a = Rate Constant of Association
 k_{-a} = Rate Constant of Disassociation

$Ag_{(Tg)} - B^{in}Ab_{(m)} + Streptavidin_{C.W.} \Rightarrow Immobilized\ complex\ (IC)$
 $Streptavidin_{C.W.}$ = Streptavidin immobilized on well
 $Immobilized\ complex\ (IC)$ = Ag-Ab bound to the well

After a suitable incubation period, the antibody-antigen bound fraction is separated from unbound antigen by decantation or aspiration. Another antibody (directed at a different epitope) labeled with an enzyme is added. Another interaction occurs to form an enzyme labeled antibody-antigen-biotinylated-antibody complex on the surface of the wells. Excess enzyme is washed off via a wash step. A suitable substrate is added to produce color measurable with the use of a microplate spectrophotometer. The enzyme activity on the well is directly proportional to the native 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.



$EnzAb_{(Tg)}$ = Enzyme labeled Antibody (Excess Quantity)
 $EnzAb_{(Tg)} - IC$ = Antigen-Antibodies Complex
 k_b = Rate Constant of Association
 k_{-b} = Rate Constant of Disassociation

4.0 REAGENTS

Materials Provided:

A. Thyroglobulin Calibrators – 1.0 ml/vial – Icons A - F

Six (6) vials of references for Thyroglobulin antigen (Tg) at levels of **0(A), 2.0 (B), 10.0(C), 40(D), 100(E), and 250(F) ng/ml**. A preservative has been added.

Note: There is no known, internationally accepted thyroglobulin standard available. The Tg used in the serum based calibrators is a highly purified (98+% pure) human Tg preparation that is calibrated gravimetrically against the reference material obtained from Community Bureau of Reference # CRM 457.

B. x-Tg Biotin Reagent – 13ml/vial – Icon ▽

One (1) vial contains biotinylated anti-Tg monoclonal mouse IgG in buffer, dye, and preservative. Store at 2-8°C.

C. Tg Enzyme Reagent – 13 ml/vial – Icon ⊞

One (1) vial contains anti-thyroglobulin IgG labeled with horseradish peroxidase (HRP) in buffer, dye, and preservative. Store at 2-8°C.

D. Streptavidin Coated Plate – 96 wells – Icon ↓

One 96-well (creat well modules) microplate coated with streptavidin and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

E. Wash Solution Concentrate – 20 ml/vial – Icon ♣

One (1) vial contains surfactant in buffered saline. A preservative has been added. Store at 2-8°C.

F. Substrate Reagent – 12ml/vial – Icon S^N

One (1) vial contains tetramethylbenzidine (TMB) and hydrogen peroxide (H₂O₂) in buffer. Store at 2-8°C.

G. Stop Solution – 8ml/vial – Icon ⊖

One (1) vial contains a strong acid (0.5M H₂SO₄). 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:

- Pipette(s) capable of delivering 0.050ml (50µl) and 0.100ml (100µl) volumes with a precision of better than 1.5%.
- Dispenser(s) for repetitive deliveries of 0.100ml (100µl) and 0.350ml (350µl) volumes with a precision of better than 1.5%
- Microplate washer or a squeeze bottle (optional).
- Microplate Reader with 450nm and 620nm wavelength absorbance capability
- Absorbent Paper for blotting the microplate wells.
- Plastic wrap or microplate cover for incubation steps.
- Vacuum aspirator (optional) for wash steps.

- Timer.
- Storage container for storage of wash buffer.
- Distilled or deionized water.
- 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 or gel barrier. Allow the blood to clot. Centrifuge the specimen to separate the serum from the cells.

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

Samples may be refrigerated at 2-8°C for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20°C for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.100ml (100µl) of the specimen is required.

7.0 QUALITY CONTROL

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

8.0 REAGENT PREPARATION

1. Wash Buffer

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

Note: Do not use reagents that are contaminated or have bacteria growth.

9.0 TEST PROCEDURE (Time 4hr 15min)

Before proceeding with the assay, bring all reagents, serum reference calibrator and controls to room temperature (20-27°C).

****Test Procedure should be performed by a skilled individual or trained professional****

- Format the microplates' wells for each calibrator, control and patient sample to be assayed in duplicate. **Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.**
- Pipette 0.050 ml (50µl) of the appropriate calibrators, controls and samples into the assigned wells.
- Add 0.100 ml (100µl) of the x-Tg Biotin Reagent to each well. **It is very important to dispense all reagents close to the bottom of the microwell.**

- Swirl the microplate gently for 20-30 seconds to mix. Cover with a plastic wrap or microplate cover.
- Incubate for 2 hours at room temperature.
- Discard the contents of the microplate by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper.
- 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 used, fill each well to the top by squeezing the container (Avoiding air bubbles). Decant the wash and repeat two (2) additional times.**
- Add 0.100 ml (100µl) of Tg Enzyme Reagent to all wells **DO NOT SHAKE THE PLATE AFTER ENZYME ADDITION**
- Cover with a plastic wrap. Incubate at room temperature for 120 minutes.
- Discard the contents of the microplate by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper.
- 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 used, fill each well to the top by squeezing the container (Avoiding air bubbles). Decant the wash and repeat two (2) additional times.**
- Add 0.100 ml (100 µl) of substrate to all wells. **Always add reagents in the same order to minimize reaction time differences between wells.**
DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION
- Cover with a plastic wrap or microplate cover. Incubate at room temperature for 15 minutes.
- 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.**
- 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 fifteen (15) minutes of adding the stop solution.**

9.1 ALTERNATE PROCEDURE (Time 2hr 15min)

This procedure can be used with the help of a laboratory hematology shaker.

- Format the microplates' wells for each calibrator, control and patient sample to be assayed in duplicate. **Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.**
- Pipette 0.050 ml (50µl) of the appropriate calibrators, controls and samples into the assigned wells.
- Add 0.100 ml (100µl) of the biotin labeled monoclonal antibody to each well. **It is very important to dispense all reagents close to the bottom of the microwell and swirl to mix.**
- Incubate at room temperature for 1 hour while shaking constantly on a hematology shaker at 150 RPM.
- Discard the contents of the microplate by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper.
- 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 used, fill each well to the top by squeezing the container (Avoiding air bubbles). Decant the wash and repeat two (2) additional times.**
- Add 0.100 ml (100µl) of Tg Enzyme Reagent to all wells
- Incubate at room temperature for 1 hour while shaking constantly on a hematology shaker at 150 RPM.
- Repeat steps 5-6 as described in the "Test Procedure" above.
- Follow steps 11-14 to develop color and measure.

10.0 CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of human thyroglobulin (Tg) in unknown specimens.

- Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.
- Plot the absorbance for each duplicate serum reference versus the corresponding Tg concentration in ng/ml on linear graph

paper (do not average the duplicates of the serum references before plotting).

- Draw the best-fit curve through the plotted points.
- To determine the concentration of Tg 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 (0.424) intersects the dose response curve at 25.2 ng/ml Tg concentration (See Figure 1).

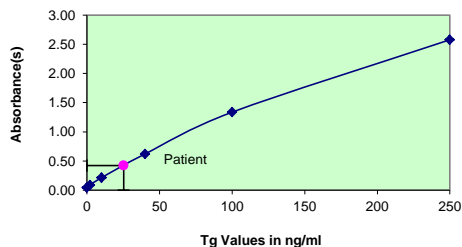
Note: Computer data reduction software designed for ELISA assays may also be used for the data reduction. **If such software is utilized, the validation of the software should be ascertained.**

EXAMPLE 1

(Data is based on 9.0 Test Procedure - time 4 hr. 15min)

Sample I.D.	Well Number	Abs (A)	Mean Abs (B)	Value (ng/ml)
Cal A	A1	0.047	0.047	0
	B1	0.047		
Cal B	C1	0.093	0.091	2
	D1	0.090		
Cal C	E1	0.221	0.217	10
	F1	0.214		
Cal D	G1	0.612	0.625	40
	H1	0.634		
Cal E	A2	1.343	1.339	100
	B2	1.335		
Cal F	C2	2.596	2.577	250
	D2	2.557		
Cont 1	E2	0.142	0.146	4.99
	F2	0.150		
Cont 2	G2	1.622	0.876	125.0
	H2	1.566		
Patient 1	A3	0.426	0.424	25.2
	B3	0.422		

Figure 1



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

11.0 Q. C. PARAMETERS

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

- The absorbance (OD) of calibrator A should be ≤ 0.06 .
- The absorbance (OD) of calibrator F should be ≥ 1.3 .
- 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

- It is important that the time of reaction in each well is held constant to achieve reproducible results.

- Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
- Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
- If more than one (1) plate is used, it is recommended to repeat the dose response curve.
- The addition of substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. The substrate and stop solution should be added in the same sequence to eliminate any time-deviation during reaction.
- Plate readers measure vertically. Do not touch the bottom of the wells.
- Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
- Use components from the same lot. No intermixing of reagents from different batches.
- Patient samples with thyroglobulin concentrations above 250 ng/ml may be diluted with the zero calibrator and re-assayed. Multiply the value obtained by the dilution factor to obtain the corrected value.
- 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.
- 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.
- 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.
- 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

- Measurements and interpretation of results must be performed by a skilled individual or trained professional.
- 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.
- The reagents for the test system have been formulated to eliminate maximal interference; however, potential interaction between rare serum specimens and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for all kinds of Immunoassays (Boscato LM, Stuart MC "Heterophilic antibodies: a problem for all immunoassays" *Clin. Chem* (1988:3427-33). For diagnostic purposes, the results from this assay should be in combination with clinical examination, patient history, and all other clinical findings.
- For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
- 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**.
- 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 RANGE OF VALUES

Based on the clinical data gathered by Monobind in concordance with the published literature a normal range was established.

Table 1: Expected Values for Tg	
POPULATION	RANGE
Adult	3.5 – 56 ng/ml

Tg is found to be elevated in patients with thyroid follicular and papillary carcinoma, thyroid adenoma, subacute thyroiditis, Hashimoto's thyroiditis and Graves' disease. Low levels of Tg are an indication of thyrotoxicosis factitia.

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

method with a population indigenous to the area in which the laboratory is located.

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

The within and between assay precisions of the Thyroglobulin 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 Tables 2 and 3.

TABLE 2				
Within Assay Precision (Values in ng/ml)				
Sample	N	X	σ	C.V.
Pool 1	22	6.2	0.41	6.6%
Pool 2	22	64.4	2.23	3.6%
Pool 3	22	194.1	8.17	4.2%

TABLE 3				
Between Assay Precision* (Values in ng/ml)				
Sample	N	X	σ	C.V.
Pool 1	10	5.8	0.52	9.0%
Pool 2	10	62.2	3.82	6.1%
Pool 3	10	192.3	10.90	5.7%

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

14.2 Sensitivity

The analytical 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 0.44 ng/ml.

14.3 Accuracy

The Tg AccuBind® ELISA test system was compared with a reference coated tube radioimmunoassay (IRMA) assay. Biological specimens from symptomatic and asymptomatic populations were used. The data obtained is displayed in Table 4.

TABLE 4			
Method	Mean (x)	Least Square Regression Analysis	Correlation Coefficient
Monobind	13.6	$y = 2.55 + 0.908(x)$	0.975
Reference	11.4		

The least square regression equation and correlation coefficient indicates excellent method agreement.

14.4 Specificity

The cross-reactivity of the Tg AccuBind® ELISA method 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 Thyroglobulin needed to produce the same absorbance.

Substance	Concentration	Cross Reactivity
Thyroglobulin	100 ng/ml	100.0%
Triiodothyronine	1000 ng/dl	N/D
Thyroxine	1000 ng/ml	N/D
TBG	100 ng/ml	N/D

14.5 High Dose Effect

Since the assay is sequential in design, high concentrations of Tg do not show the hook effect. Samples with concentrations over 50,000 ng/ml demonstrated extremely high levels of absorbance.

15.0 REFERENCES

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- Ladenson PW, "Optimal laboratory testing for diagnosis and monitoring of thyroid nodules, goiter, and thyroid cancer", *Clin Chem*, 42, 183-187 (1996).
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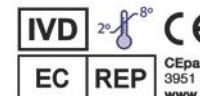
Effective Date: 2019-Jul-16 Rev. 4 DCO: 1353
MP2225 Product Code: 2225-350

Size	96(A)	192(B)	
Reagent (fill)	A)	1ml set	1ml set
	B)	1 (13ml)	2 (13ml)
	C)	1 (13ml)	2 (13ml)
	D)	1 plate	2 plates
	E)	1 (20ml)	1 (20ml)
	F)	1 (12ml)	2 (12ml)
	G)	1 (8ml)	2 (8ml)

For Orders and Inquires, please contact

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

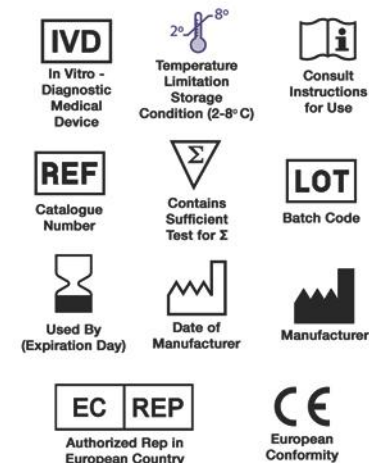
Tel: +1 949.951.2655 Mail: info@monobind.com
Fax: +1 949.951.3539 Fax: www.monobind.com



CEpartner4U, Esdoornlaan 13
3951 DBMaarn, The Netherlands
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Glossary of Symbols (EN 980/ISO 15223)





Thyrotropin (TSH) Test System Product Code: 325-300

1.0 INTRODUCTION

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

2.0 SUMMARY AND EXPLANATION OF THE TEST

Measurement of the serum concentration of thyrotropin (TSH), a glycoprotein with a molecular weight of 28,000 Daltons and secreted from the anterior pituitary, is generally regarded as the most sensitive indicator available for the diagnosis of primary and secondary (pituitary) hypothyroidism.^{1,2} The structure of human TSH is similar to that of the pituitary and placental gonadotropins, consisting of an 89-amino acid α -subunit which is similar or identical between these hormones and a 115-amino acid β -subunit, which apparently confers hormonal specificity. The production of the 2 subunits is separately regulated with apparent excess production of the α -subunit. The TSH molecule has a linear structure consisting of the protein core with carbohydrate side chains; the latter accounts for 16% of the molecular weight.

TSH measurements are equally useful in differentiating secondary and tertiary (hypothalamic) hypothyroidism from the primary thyroid disease. TSH release from the pituitary is regulated by thyrotropin releasing factor (TRH), which is secreted by the hypothalamus, and by direct action of T4 and triiodothyronine (T3), the thyroid hormones, at the pituitary. Increase levels of T3 and T4 reduces the response of the pituitary to the stimulatory effects of TRH. In secondary and tertiary hypothyroidism, concentrations of T4 are usually low and TSH levels are generally low or normal. Either pituitary TSH deficiency (secondary hypothyroidism) or insufficiency of stimulation of the pituitary by TRH (tertiary hypothyroidism) causes this. The TRH stimulation test differentiates these conditions. In secondary hypothyroidism, TSH response to TRH is blunted while a normal or delayed response is obtained in tertiary hypothyroidism.

Further, the advent of immunoassay methods has provided the laboratory with sufficient sensitivity to enable the differentiating of hyperthyroidism from euthyroid population and extending the usefulness of TSH measurements. This method is a second-generation assay, which provides the means for discrimination in the hyperthyroid-euthyroid range. The functional sensitivity (<20% between assay CV) of the one-hour procedure is 0.195 μ U/ml while the two-hour procedure has a functional sensitivity of 0.095 μ U/ml.³

In this method, TSH calibrator, patient specimen or control is first added to a streptavidin coated well. Biotinylated monoclonal and enzyme labeled antibodies are added and the reactants mixed. Reaction between the various TSH antibodies and native TSH forms a sandwich complex that binds with the streptavidin coated to the well.

After the completion of the required incubation period, the antibody bound enzyme-thyrotropin conjugate is separated from

the unbound enzyme-thyrotropin 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.

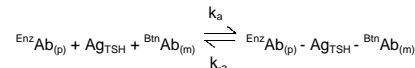
The employment of several serum references of known thyrotropin levels permits construction of a dose response curve of activity and concentration. From comparison to the dose response curve, an unknown specimen's activity can be correlated with thyrotropin concentration.

3.0 PRINCIPLE

Immunoassay (TYPE 3):

The essential reagents required for an immunoassay include high affinity and specificity antibodies (enzyme conjugated 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-TSH 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:



$\text{BiotAb}_{(m)}$ = Biotinylated Monoclonal Antibody (Excess Quantity)
 Ag_{TSH} = Native Antigen (Variable Quantity)
 $\text{EnzAb}_{(p)}$ = Enzyme-Polyclonal Antibody (Excess Quantity)
 $\text{EnzAb}_{(p)} - \text{Ag}_{\text{TSH}} - \text{BiotAb}_{(m)}$ = 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:
 $\text{EnzAb}_{(p)} - \text{Ag}_{\text{TSH}} - \text{BiotAb}_{(m)} + \text{Streptavidin}_{\text{CW}} \rightarrow \text{immobilized complex}$
 $\text{Streptavidin}_{\text{CW}}$ = Streptavidin immobilized on well
 Immobilized complex = sandwich complex bound to the well surface

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

4.0 REAGENTS

Materials Provided:

A. TSH Calibrators – 1ml/vial - Icons A-G

Seven (7) vials of references for TSH Antigen at levels of 0(A), 0.5(B), 2.5(C), 5.0(D), 10(E), 20(F) and 40(G) μ U/ml. Store at 2-8°C. A preservative has been added.

Note: The calibrators, human serum based, were calibrated using a reference preparation, which was assayed against the WHO 2nd IRP 80/558.

B. TSH Enzyme Reagent – 13ml/vial - Icon $\text{\textcircled{E}}$

One (1) vial containing enzyme labeled affinity purified polyclonal goat antibody, biotinylated monoclonal mouse IgG in buffer, dye, and preservative. Store at 2-8°C.

C. Streptavidin Coated Plate – 96 wells - Icon $\text{\textcircled{J}}$

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/ml - Icon $\text{\textcircled{L}}$

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 $\text{\textcircled{S}}$

One (1) vial containing tetramethylbenzidine (TMB) in buffer. Store at 2-8°C.

F. Substrate B – 7ml/vial - Icon $\text{\textcircled{B}}$

One (1) vial containing hydrogen peroxide (H_2O_2) in buffer. Store at 2-8°C.

G. Stop Solution – 8ml/vial - Icon $\text{\textcircled{STOP}}$

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:

- Pipette(s) capable of delivering 0.050ml (50 μ l) and 0.100ml (100 μ l) volumes with a precision of better than 1.5%.
- Dispenser(s) for repetitive deliveries of 0.100ml (100 μ l) and 0.350ml (350 μ l) volumes with a precision of better than 1.5% (optional).
- Microplate washer or a squeeze bottle (optional).
- Microplate Reader with 450nm and 620nm wavelength absorbance capability.
- Absorbent Paper for blotting the microplate wells.
- Plastic wrap or microplate cover for incubation steps.
- Vacuum aspirator (optional) for wash steps.
- Timer.
- Storage container for storage of wash buffer.
- Distilled or deionized water.
- 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 redtop venipuncture tube without additives or gel barrier. Allow the blood to clot. Centrifuge the specimen to separate the serum from the cells.

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

Samples may be refrigerated at 2-8°C for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20°C for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, (100 μ l) 0.100 ml 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. The individual laboratory should set acceptable assay performance limits. Other parameters that should be monitored include the 80, 50 and 20% intercepts of the dose response curve for run-to-run reproducibility. 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

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

Note1: Do not use the working substrate if it looks blue.

Note 2: Do not use reagents that are contaminated or have bacteria growth.

9.0 TEST PROCEDURE

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

- 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.**
- Pipette 0.050 ml (50 μ l) of the appropriate serum reference, control or specimen into the assigned well.
- Add 0.100 ml (100 μ l) of the TSH Enzyme Reagent to each well. **It is very important to dispense all reagents close to the bottom of the coated well.**
- Swirl the microplate gently for 20-30 seconds to mix and cover.
- Incubate 60 minutes at room temperature. **
- Discard the contents of the microplate by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper.
- Add 0.350ml (350 μ l) 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.**
- Add 0.100 ml (100 μ l) 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**
- Incubate at room temperature for fifteen (15) minutes.
- Add 0.050ml (50 μ l) 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.**
- 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.**

** For better low-end sensitivity (< 0.5 μ U/ml), incubate 120 minutes at room temperature. The 40 μ U/ml calibrator should be excluded since absorbance over 3.0 units will be experienced. Follow the remaining steps.

Note: Dilute samples reading over 40 μ U/ml by 1:5 and 1:10 with TSH '0' Calibrator. Multiply the results by the dilution factor to obtain accurate results.

10.0 CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of thyrotropin in unknown specimens.

- Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1
- Plot the absorbance for each duplicate serum reference versus the corresponding TSH concentration in μ U/ml on linear graph paper (do not average the duplicates of the serum references before plotting).

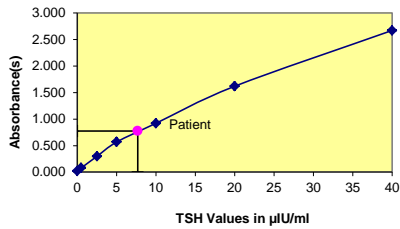
- Draw the best-fit curve through the plotted points.
- To determine the concentration of TSH for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in $\mu\text{IU/ml}$) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance (0.775) intersects the dose response curve at (7.66 $\mu\text{IU/ml}$) TSH concentration (See Figure 1).

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

EXAMPLE 1

Sample I.D.	Well Number	Abs	Mean Abs	Value ($\mu\text{IU/ml}$)
Cal A	A1	0.018	0.019	0
	B1	0.021		
Cal B	C1	0.076	0.079	0.5
	D1	0.082		
Cal C	E1	0.302	0.298	2.5
	F1	0.293		
Cal D	G1	0.556	0.567	5.0
	H1	0.577		
Cal E	A2	0.926	0.921	10
	B2	0.916		
Cal F	C2	1.610	1.619	20
	D2	1.629		
Cal G	E2	2.694	2.671	40
	F2	2.647		
Control	G2	0.800	0.775	7.66
	H2	0.751		
Patient	A3	1.391	1.383	16.65
	B3	1.375		

Figure 1



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

11.0 Q.C. PARAMETERS

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

- The absorbance of calibrator 'G' (40 $\mu\text{IU/ml}$) should be ≥ 1.3 .
- Four out of six quality control pools should be within the established ranges.

12.0 RISK ANALYSIS

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

12.1 Assay Performance

- It is important that the time of reaction in each well is held constant to achieve reproducible results.
- Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
- Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
- If more than one (1) plate is used, it is recommended to repeat the dose response curve.
- 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.

- Plate readers measure vertically. Do not touch the bottom of the wells.
- Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
- Use components from the same lot. No intermixing of reagents from different batches.
- 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.
- Patient specimens with TSH concentrations over 40 $\mu\text{IU/ml}$ may be diluted (1:5 or 1:10) with the '0' calibrator and re-assayed. The sample's concentration is obtained by multiplying the result by the dilution factor.
- 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.
- 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.
- Risk Analysis- as required by CE Mark IVD Directive 98/79/EC - for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com.

12.2 Interpretation

1. Measurement and interpretation of results must be performed by a skilled individual or trained professional.

- 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.
- The reagents for the test system have been formulated to eliminate maximal interference; however, potential interaction between rare serum specimens and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for all kinds of immunoassays (Boscato LM, Stuart MC. 'Heterophilic antibodies: a problem for all immunoassays' Clin. Chem. 1988:3427-33). For diagnostic purposes, the results from this assay should be in combination with clinical examination, patient history and all other clinical findings. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
- 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.**
- 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.
- Serum TSH concentration is dependent upon a multiplicity of factors: hypothalamus gland function, thyroid gland function, and the responsiveness of pituitary to TRH. **Thus, thyrotropin concentration alone is not sufficient to assess clinical status.**

- Serum TSH values may be elevated by pharmacological intervention. Domperidone, amiodazon, iodide, phenobarbital, and phenytoin have been reported to increase TSH levels.
- A decrease in thyrotropin values has been reported with the administration of propranolol, methimazol, dopamine and d-thyroxine.⁴
- Genetic variations or degradation of intact TSH into subunits may affect the binding characteristics of the antibodies and influence the final result. Such samples normally exhibit different results among various assay systems due to the reactivity of the antibodies involved.

"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 TSH AccuBind® ELISA Test System. The number and determined range are given in Table 1. A nonparametric method (95% Percentile Estimate) was used.

TABLE 1 Expected Values for the TSH ELISA Test System (in $\mu\text{IU/ml}$)		
Number	139	2.5 Percentile-70% Conf Int
Low Normal	0.39	Low Range 0.28 – 0.53
High Normal	6.16	High Range 5.60 – 6.82

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 TSH AccuBind® test system were determined by analyses on three different levels of pool control sera. The number (N), mean (X) value, 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 $\mu\text{IU/ml}$)

Sample	N	X	σ	C.V.
Pool 1	24	0.37	0.03	8.1%
Pool 2	24	6.75	0.43	6.4%
Pool 3	24	29.30	1.94	6.6%

TABLE 3
Between Assay Precision* (Values in $\mu\text{IU/ml}$)

Sample	N	X	σ	C.V.
Pool 1	10	0.43	0.04	9.3%
Pool 2	10	6.80	0.54	7.9%
Pool 3	10	28.40	1.67	5.9%

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

14.2 Sensitivity

The sensitivity (detection limit) was ascertained by determining the variability of the 0 $\mu\text{IU/ml}$ serum calibrator and using the 2 σ (95% certainty) statistic to calculate the minimum dose:

For 1 hr incubation = 0.078 $\mu\text{IU/ml}$
For 2 hr incubation = 0.027 $\mu\text{IU/ml}$

14.3 Accuracy

The TSH AccuBind® ELISA test system was compared with a reference immunochemiluminescence assay. Biological specimens from hypothyroid, euthyroid and hyperthyroid populations were used (The values ranged from 0.01 $\mu\text{IU/ml}$ – 61 $\mu\text{IU/ml}$). The total number of such specimens was 241. The least square regression equation and the correlation coefficient were computed for the TSH 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
Monobind	4.54	$y = 0.47 + 0.968 (x)$	0.995
Reference	4.21		

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

14.4 Specificity

The cross-reactivity of the TSH AccuBind® ELISA test system to selected substances was evaluated by adding the interfering substance to a serum matrix at various concentrations. The cross-reactivity was calculated by deriving a ratio between dose of interfering substance to dose of thyrotropin needed to produce the same absorbance.

Substance	Cross Reactivity	Concentration
Thyrotropin (hTSH)	1.0000	-
Foliotropin (hFSH)	< 0.0001	1000ng/ml
Lutropin Hormone (hLH)	< 0.0001	1000ng/ml
Chorionic Gonadotropin (hCG)	< 0.0001	1000ng/ml

14.5 Correlation between 1 hr and 2 hr incubation

The one- (1) hr and two (2) hr (optional) incubation procedures were compared. Thirty (30) biological specimens (ranging from 0.1 – 18.5 $\mu\text{IU/ml}$) were used. The least square regression equation and the correlation coefficient were computed for the 2 hr procedure (y) in comparison with the 1 hr method (x). Excellent agreement is evidenced by the correlation coefficient, slope and intercept: $Y = 0.986 (x) + 0.119$ Regression Correlation = 0.998

15.0 REFERENCES

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Size	96(A)	192(B)	480(D)	960(E)
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)

For Orders and Inquiries, please contact

Monobind Inc.
100 North Pointe Drive
Lake Forest, CA 92530 USA

Tel: +1 949.951.2685 Mail: info@monobind.com
Fax: +1 949.951.3539 Fax: www.monobind.com



CEPartner4U, Esdoornlaan 13
3951 DE Masmr, The Netherlands
www.cepartner4u.eu

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