Monobind Inc.
Lake Forest, CA 92630, USA

AccuBind
ELISA Microwells

Rapid β-Human Chorionic Gonadotropin (Rapid - hCG)

Test System
Product Code: 3325-300

1.0 INTRODUCTION

Intended Use: The Visual Qualitative or Spectrophotometric Semi-Semi-quantitative Determination of hCG in Human Serum or Urine by a Microplate Immunoenzymometric assay for early detection of pregnancy.

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.

Mishell *et al*,[4] reported data obtained by immunologic measurements of hCG in urine and serum obtained throughout pregnancy. Values for serum or urine (voided in the morning) were similar. There was a rapid rise in the hCG levels after conception that peaked between 60-80 days of pregnancy, with a mean value of 100,000 mIU/ml, followed by a gradual fall to 20,000 mIU/ml by day 120.

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

Sandwich Equlibrium ELISA Method (Type 2):
The essential reagents required for an immunoenzymometric assay include high affinity and specificity antibodies (signal and capture), with different and distinct epitope recognition, **in excess,** and native antigen. In this procedure, the calibrator, control or patient sample is added to the wells coated with anti HCG antibody. HCG from the sample binds to the Anti-HCG (MoAb) on the wells. Subsequently an enzyme labeled Anti-HCG is added to the wells. HCG from the sample forms a sandwich between the two antibodies. Excess enzyme and sample is removed via a wash step. The interaction is illustrated by the following equation:

$$Enz_{Ab_{(p)}} + Ag_{hCG} + Ab_{(m)} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} Enz_{Ab_{(p)}}\text{-}Ag_{hCG}\text{-}Ab_{(m)}$$

$Ab_{(m)}$ = Anti-HCG (MoAb)(On the Microwells in Excess Quantity)

Ag_{hCG} = Native Antigen (Variable Quantity)

$Enz_{Ab_{(hCG)}}$=Enzyme labeled Goat α hCG (P) (Excess Quantity)

$Enz_{Ab_{(hCG)}}\text{-}Ag_{hCG}\text{-}Ab_{(m)}$=Ag-Antibodies Sandwich complex

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

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

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

4.0 REAGENTS

Materials Provided:

A. hCG Calibrators – 1ml/vial - Icons A-E (Lyophilized) (A-E)
Five (5) vials, of references for hCG Antigen at levels of 0(**A**), 25(**B**), 50(**C**), 100(**D**) and 250(**E**) mIU/ml. Store at 2-8°C. Reconstitute each vial with 1.0ml of distilled or deionized water.
Note: The calibrators, human serum based, were calibrated using a reference preparation, which was assayed against the WHO 3rd IS (75/537).

B. Anti-hCG Enzyme Conjugate – 13 ml/vial
One (1) vial, containing enzyme labeled affinity purified Goat Anti-HCG (IgG) in buffer, dye, and preservative. Store at 2-8°C.

C. Anti-HCG Coated Microplate – 96 wells.
One 96-well microplate coated with Anti-HCG (MoAb-IgG) and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

D. Substrate Reagent – 14 ml/vial
One (1) bottle, containing tetramethylbenzidine (TMB) and H_2O_2 in a buffer. Store at 2-8°C.

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

F. Stop Solution – 8 ml/vial
One (1) bottle, containing 1N HCl. Store at 2-8°C.

G. Product Insert

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

4.1 Required But Not Provided:
1. Pipets capable of delivering 25µl, 50µl and 100µl volumes with a precision better than 1.5%.
2. Plastic wrap or microplate cover for incubation steps.
3. Microplate Reader with 450nm and 620nm wavelength absorbance capability. (*For Semi-Semi-quantitative Interpretation Only)*
4. Absorbent Paper for blotting the microplate wells.
5. Timer.
6. Quality control materials.
7. Urine collection containers.

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

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

Urine Sample:
Collect urine sample in a clean container. For most accurate results it is advisable to collect first morning urine 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 of the specimen is required.

7.0 QUALITY CONTROL

Each laboratory should assay controls at levels in the low, medium 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. 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 AND STORAGE:

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.
Note1 : Do not use reagents that are contaminated or have bacteria growth.

9.0 TEST PROCEDURE

Before proceeding with the assay, bring all reagents, references controls and samples 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.
   Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C
2. Dispense pipette 0.025 ml (25µl) of the appropriate serum reference, control or specimen into the assigned well.
3. Add 100µl of hCG-Enzyme Conjugate solution to all wells.
4. Swirl the microplate gently for 5-10 seconds to mix and incubate at room temperature for 10 minutes.
5. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.
6. 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 used, fill each well to the top by squeezing the container. Avoiding air bubbles. Decant the wash and repeat two (2) additional times.**
7. Add 100µl of substrate solution to all the wells.
   DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION
8. Incubate at room temperature for 5 minutes.
9. For ***Qualitative results*** see Interpretation of Results Section.
10. For ***Semi-quantitative results*** go to step# 11 below.
11. Add 50µl of stop solution to each well and gently mix.
12. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) in a microplate reader.

Note: The results should be read within thirty (30) minutes of adding the stop solution.

10.0 INTERPRETATION OF RESULTS

Qualitative Results:
1. Do not add the stop solution to the wells. Blue color is much easier to interpret than the yellow color that shows after the addition of stop solution.
2. Compare the blue color, in the sample well, to the color in the well assigned to Calibrator 'B' (*25 mIU/ml*).
3. If the color in the sample well is less than the color in the Calibrator 'B' well the sample should be considered ***Negative*** because it has hCG concentration < 25 mIU/ml at the time the sample was taken.
4. If the color in the sample well is more than the color in the Calibrator 'B' well, the sample should be considered ***Positive*** because it has hCG concentration > 25 mIU/ml at the time the sample was taken.

EXAMPLE 1

Sample I.D.	Well Number	Absorbance 450 nm	Results	
			Semi-quantitative (mIU/ml)	Qualitative Visual
Cal A	A1	0.029	0	
Cal B	B1	0.373	25	
Cal C	C1	0.646	50	
Cal D	D1	1.097	100	
Cal E	E1	1.654	250	
Serum Sample	F1	0.088	***4.3***	***Negative***
Urine Sample	G1	0.661	***51.8***	***Positive***

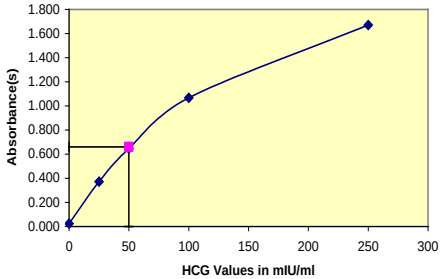
* Visual interpretation (***Qualitative Determination***) of results is based on the blue color reading before stopping the reaction with stop solution. See '*Interpretation of Results*'.

*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.

Semi-quantitative Results:
A dose response curve is used to ascertain the concentration of human chorionic gonadotropin 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 serum reference versus the corresponding hCG concentration in mIU/ml on linear graph paper.
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 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. (See Figure 1 above).

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.

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

12.0 RISK ANALYSIS

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

12.1 Assay Performance

1. ***The Monobind AccuBind™ Rapid HCG Microwell Elisa test is intended to be used for early determination of pregnancy and should not be used for monitoring of advanced pregnancy. Monobind AccuBind™ HCG Elisa (Cat# 825-300) should be used for that purpose.***
2. It is important that the time of reaction in each well is held constant to achieve reproducible results.
3. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
4. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
5. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
6. 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.
7. Plate readers measure vertically. Do not touch the bottom of the wells.
8. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
9. Use components from the same lot. No intermixing of reagents from different batches.
10. For semi-quantitative determination the patient specimens with hCG concentrations above 250 mIU/ml may be diluted with normal male serum (hCG < 1 mIU/ml) or normal male urine and re-assayed. The sample's concentration is obtained by multiplying the result by the dilution factor.
11. 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.
12. 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.
13. 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.
14. Risk Analysis- as required by CE Mark IVD Directive 98/79/EC - for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com.

12.2 Interpretation

1. ***Measurements and interpretation of results must be performed by a skilled individual or trained professional.***
2. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.
3. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
4. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, Monobind shall have no liability.
5. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.
6. False positive results may occur in the presence of a wide variety of trophoblastic and nontrophoblastic tumors that secrete hCG. Therefore, the possibility of an hCG secreting neoplasia should be eliminated prior to diagnosing pregnancy.
7. Also, false positive results may be seen when assaying specimens from individuals taking the drugs Pergonal* and Clomid**. Additionally Pergonal will often be followed with an injection of hCG.
8. Spontaneous micro abortions 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 (4, 5, 6).
9. Following therapeutic abortion, detectable hCG may persist for as long as three to four weeks. The disappearance rate of hCG, after spontaneous abortion, will vary depending upon the quantity of viable residual trophoblast (4, 5, 6, 7).

*Pergonal 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 (*males and non-pregnant* females) population was undertaken to determine expected values for the Accubind™ Rapid hCG ELISA Microplate Test System. The data shows that a serum or urine sample from a normal healthy male or non-pregnant female should read ≤ 5.0 mIU/ml.

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

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

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

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 Accubind™ Rapid hCG ELISA Microplate Test System were determined by analyses on three different levels of control sera. The number, mean value, standard deviation and coefficient of variation for each of these control sera are presented in Table 3 and Table 4.

TABLE 3
Within Assay Precision (Values in mIU/ml)

Sample	N	X	σ	C.V.
Level 1	20	5.5	0.35	6.4%
Level 2	20	20.5	0.95	4.6%
Level 3	20	91.6	7.07	7.7%

TABLE 4
Between Assay Precision* (Values in mIU/ml)

Sample	N	X	σ	C.V.
Level 1	10	6.1	0.52	8.6%
Level 2	10	22.3	1.63	7.3%
Level 3	10	85.1	6.17	7.3%

*As measured in ten experiments in duplicate.

14.2 Sensitivity

The Accubind™ Rapid hCG ELISA Microplate Test System has a sensitivity of 2.5 mIU/ml.

14.3 Accuracy

The Accubind™ Rapid hCG ELISA Microplate Test System was compared with a predicate Elisa immunoassay. Biological specimens from normal and pregnant populations were assayed. The total number of such specimens was 244. 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 in Table 4.

TABLE 4

Method	Mean (x)	Least Square Regression Analysis	Correlation Coefficient
This Method	25.57	y = 1.0135+0.9474x	0.956
Predicate	24.44		

Only slight amounts of bias between the Accubind™ hCG ELISA Microplate 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 Accubind™ Rapid hCG ELISA Microplate 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 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
Thyrotropin (TSH)	< 0.0001	1000ng/ml

14.5 High Dose Hook Effect

The Monobind Accubind™ Rapid hCG ELISA Microplate Test System will detect samples with hCG concentrations up to 25,000 mIU/ml as positive qualitatively.

15.0 REFERENCES

1. Kosasa T.S., "Measurement of Human Chorionic Gonadotropin." *Journal of Reproductive Medicine* **26,** 201-6. (1981)
2. Danzer H., Braunstein G.D., et al., "Maternal Serum Human Chorionic Gonadotropic Concentrations and Fetal Sex Predictions." *Fertility and Sterility* **34,** 336-40. (1980)
1. Braunstein G.D., et at., "Serum Human Chorionic Gonadotropin Levels through Normal Pregnancy ", *American Journal of Obstetrics and Gynecology* **126**, 678-81. (1976)
2. Mishell D.R., et al.: "HCG concentrations in urine and serum during pregnancy" *J.Clin. Endocrinology*. **23,** 125. (1963)
4. Goldstein D.P., and Kosasa T.S., "The Subunit Radioimmunoassay for HCG Clinical Application." *Gynecology* **6,** 145-84. (1975)
5. Batzer F. "Hormonal Evaluation of Early Pregnancy", *Fertility and Sterility* **34,** 1-12. (1980)
6. Braunstein, G.D., et al., "First-Trimester Chorionic Gonadotropin Measurements as an Aid to the Diagnosis of Early Pregnancy Disorders", *American Journal of Obstetrics and Gynecology* **131,** 25-32. (1978)
7. Lenton E., Neal L. and Sulaiman R., "Plasma Concentrations of Human Gonadotropin from the time of Implantation until the Second Week of Pregnancy *", Fertility and Sterility* **37,** 773-78. (1982)
8. Cole LA., "Immunoassay of hCG, its free subunits and metabolites." *Clin. Chem*. **43,** 2233-43. (1997)
9. Stenman U-H, Unkilla Kalilo L., Korhonen J., Alfthan H. "Immunoprocedures for detecting human chorionic gonadotropin; clinical aspects and doping control." *Clin Chem.* **43,** 1293-8. (1997)
10. Rotmensch S., Cole LA. "False diagnosis and needless therapy of presumed malignant disease in womenwith false positive human chorionic gonadotropin concentrations." *Lancet* **355,** 712-15. (2000)

Revision: 3 **Date: 060712** **DCO: 0653**
Cat #: *3325-300*

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 (14ml)	2 (14ml)
	F)	1 (8ml)	2 (8ml)

For Orders and Inquiries, please contact

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

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

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