Monobind Inc.
Lake Forest, CA 92630, USA

AccuLite
CLIA Microwells

β-Human Chorionic Gonadotropin Extended Range (hCG-XR) Test System

Product Code: 8875-300

1.0 INTRODUCTION

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

2.0 SUMMARY AND EXPLANATION OF THE TEST

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

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

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

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

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

3.0 PRINCIPLE

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

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

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

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

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

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

4.0 REAGENTS

Materials Provided:

A. hCG-XR Calibrators – 1 ml/vial – Icons A-F
Six (6) vials of serum reference calibrators for hCG Antigen at levels of 0 (**A**), 5 (**B**), 25 (**C**), 100 (**D**), 250 (**E**) and 1000 (**F**) mIU/ml. Store at 2-8 °C
Note: The calibrators, human serum based, were calibrated using a reference preparation, which was assayed against the WHO 3rd IS (75/537).

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

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

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

E. Signal Reagent A – 7ml/vial – Icon C[A]
One (1) vial containing luminol in buffer. Store at 2-8 °C.

F. Signal Reagent B – 7ml/vial – Icon C[B]
One (1) vial containing hydrogen peroxide (H_2O_2) in buffer. Store at 2-8 °C.

G. 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 enough for a single 96-well microplate assay.

4.1 Required But Not Provided:
1. Pipets capable of delivering 0.025 ml (25 µl), 0.050 ml (50 µl) and 0.100 ml (100 µl) volumes with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100 ml (100 µl) and 0.350 ml (350 µl) volumes with a precision of better than 1.5%.
3. Microplate washers or a squeeze bottle (optional).
4. Microplate Luminometer
5. Test tube(s) for mixing signal reagents A & B.
6. Absorbent Paper for blotting the microplate wells.
7. Plastic wrap or microplate covers for incubation steps.
8. Vacuum aspirator (optional) for wash steps.
9. Timer.
10. Quality control materials

5.0 PRECAUTIONS

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

All products that contain human serum have been found to be non-reactive for Hepatitis B Surface Antigen, HIV 1&2 and HCV Antibodies by FDA 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, 0.050 ml (50 µl) of the specimen is required.

7.0 QUALITY CONTROL

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

8.0 REAGENT PREPARATION

1. **Wash Buffer**
Dilute contents of wash concentrate to 1000ml (1.0µl) with distilled or deionized water in a suitable storage container. Store diluted buffer at 2-30 °C for up to 60 days.
2. **Working Signal Reagent Solution -** Store at 2-8 °C
Determine the amount of reagent needed and prepare by mixing equal portions of Signal Reagent A and Signal Reagent B in a clean container. For example, add 1 ml of A and 1ml of B per two (2) eight well strips. A slight excess of solution is made. **Discard the unused portion if not used within 36 hours after mixing.** If complete utilization of the reagents is anticipated, within the above time constraint, pour the contents of Signal Reagent B into Signal Reagent A and label accordingly.

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 references and controls to room temperature (20-27 ℃).

*****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 0.025 ml (25 µl) of the appropriate serum reference, control or specimen into the assigned well.
3. Add 0.100 ml (100 µl) of hCG-XR Tracer Reagent to all wells.
4. Swirl the microplate gently for 20-30 seconds to mix and cover.
5. Incubate 20 minutes at room temperature.
6. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.
7. Add 0.350 ml (350 µl) of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat four (4) additional times for a total of five (5) 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 four (4) additional times.**
8. Add 0.100 ml (100 µl) of working signal reagent 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 SIGNAL ADDITION
9. Incubate at room temperature in the dark for five (5) minutes.
10. Read the relative light units in each well within a chemiluminescence microplate reader for 0.5-1.0 seconds. **The results should be read within thirty (30) minutes of adding the signal solution.**

10.0 CALCULATION OF RESULTS

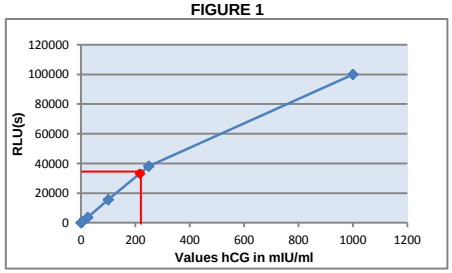
A dose response curve is used to ascertain the concentration of hCG in unknown specimens.
1. Record the RLUs obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the light intensity for each duplicate serum reference versus the corresponding hCG concentration in mIU/ml 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 RLUs of the 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 RLUs (33193) of the unknown intersects the calibration curve at 216.82 mIU/ml hCG concentration (See Figure 1).

Note: Computer data reduction software designed for CLIA assays may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained. Duplicates of the unknown may be averaged as indicated (see Figure 1).

EXAMPLE 1

Sample I.D.	Well Number	RLU (A)	Mean RLU (B)	Value (mIU/ml)
Cal A	A1	57	58	0
	B1	59		
Cal B	C1	631	618	5
	D1	604		
Cal C	E1	3777	3701	25
	F1	3625		
Cal D	G1	15763	15563	100
	H1	15363		
Cal E	A2	38334	38201	250
	B2	38067		
Cal F	C2	99441	100000	1000
	D2	100559		
Sample	E2	33537	33193	216.82
	F2	32850		

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. In addition, the RLUs of the claibrators have been normalized to 100,000 RLUs for the F calibrator (greatest light output). This conversion minimizes differences caused by efficiency of the various instruments that can be used to measure light output.

11.0 Q.C. PARAMETERS

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

1. The Dose Response Curve should be within established parameters.
2. Four out of six quality control pools should be within the established ranges.

12.0 RISK ANALYSIS

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

12.1 Assay Performance

1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. The addition of signal solution initiates a kinetic reaction; therefore, the signal reagent should be added in the same sequence to eliminate any time-deviation during reaction.
6. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
7. Use components from the same lot. No intermixing of reagents from different batches.
8. Patient specimens with hCG concentrations above 1000 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.
9. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from Monobind's IFU may yield inaccurate results.
10. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.
11. It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.
12. Risk Analysis as required by CE Mark IVD Directive 98/79/EC for this and other devices made by Monobind can be requested via email from Monobind@monobind.com.

12.2 Interpretation

1. ***Measurements and interpretation of results must be performed by a skilled individual or trained professional.***
2. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.
3. The reagents for the test system procedure have been formulated to eliminate maximal interference; however, potential interaction between rare serum specimens and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for all kinds of immunoassays. (*Boscato LM Stuart MC. 'Heterophilic antibodies: a problem for all immunoassays' Clin. Chem. 1988:3427-33).* For diagnostic purposes, the results from this assay should be used in combination with clinical examination, patient's history and, all other clinical findings.
4. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
5. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, Monobind shall have no liability.
6. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.
7. 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.
8. 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.
9. 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.[4,5,6]
10. 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]
11. **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 VALUE

A study of an apparent normal adult population was undertaken to determine expected values for the HCG XR AccuLite® CLIA Test System. The mean (X) values, standard deviations (σ) and expected ranges (±2σ) are presented in Table 1.

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

Number	50
Mean	2.3
Standard Deviation	1.6
Expected Ranges (±2σ)	0.01 – 5.5

Expected levels for hCG during normal pregnancy[3] are listed in Table 2.

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

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

Values for AFP, hCG and uE3 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 assay precision of the hCG-XR AccuLite® CLIA 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 4.

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

Sample	N	X	σ	C.V.%
Level 1	24	6.883	0.326	4.7
Level 2	24	21.357	0.616	2.9
Level 3	24	440.328	11.934	2.7

14.2 Sensitivity

The hCG-XR AccuLite® CLIA test system has a sensitivity of 0.182 mIU/ml, which is equivalent to 0.00540mIU/well. The analytical sensitivity (detection limit) was ascertained by deteremining the variability of the '0 mIU/ml' serum calibrator and using the 2σ (95% certainty) statistic to calculate the minimum dose.

14.3 Accuracy

This hCG-XR AccuLite® CLIA test system was compared with a reference enzyme immunoassay. Biological specimens from normal and pregnant populations were assayed. The total number of such specimens was 88. The least square regression equation and the correlation coefficient were computed for the hCG-XR AccuLite® CLIA in comparison with the reference method. The data obtained is displayed in Table 5.

TABLE 5

Method	Mean (x)	Least Square Regression Analysis	Correlation Coefficient
This Method	28.5	y = -1.020 + 0.98(x)	0.954
Reference	30.2		

Only slight amounts of bias between the hCG-XR AccuLite® CLIA 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-XR AccuLite® CLIA 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
hrotropin (TSH)	< 0.0001	1000ng/ml

15.0 REFERENCES

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Product Code: 8875-300 **MP8875**

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)

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

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

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

Glossary of Symbols
(EN 980/ISO 15223)

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