EnzAb(m) - AghCG - BtnAb(m)

Present on the surface of the well is quantitated by reaction with a sandwich complex that binds with the streptavidin coated to the conjugate by aspiration or decantation. The activity of the enzyme takes place during the assay at the surface of a microplate well.

According to the literature, hCG is detectable as early as 10 days after implantation, and serves to support the corpus luteum during the early weeks of pregnancy. Microplate Enzyme Immunoassay, Colorimetric

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

$$\text{EnzAb(hCG) - AghCG - BtnAb(m)} = A_{\text{Ag}-\text{Antibodies Sandwich complex}}$$

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 separated from unbound antigen by decantation or aspiration. The antibody-bound fraction is then collected in a test tube. The activity of the enzyme-chorionic gonadotropin antibody bound conjugate is then treated with streptavidin immobilized on well.

$$\text{EnzAb(x-hCG) = Enzyme labeled Antibody (Excess Quantity)}$$

$$\text{Streptavidin CW = Streptavidin immobilized on well}$$

**Materials Provided:**

- hCG Calibrators – 1 ml/vial - Icos A-F
- hCG Reagent – 13 ml/vial - Icos
- Wash Solution Concentrate – 20 ml/vial - Icos
- Kit and component stability are identified on the aluminum bag, seal and store at 2-8°C

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 reaction mixed. Reagent interaction present on the surface of the well is quantitated with a suitable substrate to produce color.

**In Vitro Diagnostic Use**

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 accurately determine whether a specimen is infected with an infectious agent, the test results should be read within thirty (30) minutes of adding the stop solution.

**5.0 PRECAUTIONS**

- 2. Pipette 0.025 ml (25uL) of the appropriate serum reference or unknown specimen into the assigned well.
- 3. Add 0.100 ml (100uL) 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 (350uL) of wash buffer (see Reagent Preparation Section), décant (tap and blot) or aspirate. Repeat for 2 additional times for a total of three (3) washes. An automatic or manual plate washer can be used. Follow the manufacturer’s instructions for generation of the washing buffer. If a squeeze bottle is used, fill each well by depressing the cap (avoiding air bubbles) to disperse the wash.
- 8. Decant the wash and repeat two additional times.
- 9. Add 0.100 ml (100uL) 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.
- 10. Incubate at room temperature for fifteen (15) minutes.
- 11. Add 0.050ml (50uL) of stop solution to each well and gently mix for 15-20 seconds. Any unused reagents should be discarded. The results should be read within thirty (30) minutes of adding the stop solution.

**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 bioassay should be ascertained.
11.0 QC. PARAMETERS
In order for the assay results to be considered valid the following criteria should be met:
1. The absorbance (OD) of calibrator F should be 2.1.
2. Four out of six quality control pools should be within the established range outlined in the screening, Prenatal Diagnosis, (1980).

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.
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 spuriously results.
8. Use components from the same lot. No intermixing of reagents from different batches.
9. Patient specimens with hCG concentrations above 250 mIU/ml may be diluted with normal male serum (hCG < 1 mIU/ml) and re-assayed. The sample’s concentration is obtained by multiplying the result by the dilution factor.
10. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from the procedure may yield inaccurate results.
11. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.

12.2 Interpretation
1. Measurements and interpretation of results must be performed by a skilled individual or trained professional.
2. Laboratory results are only intended 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. Hormetic antibodies often cause these interactions and have been known to be problems for all kinds of immunoassays (Climent, M. Stuart MC., Hormetic antibodies: a problem for all immunoassays’ Clin.

1988:3427-33). For diagnostic purposes, the results from this assay should be interpreted with clinical examination, patient history and all other clinical findings. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
4. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, Monobind shall have no liability.
5. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.
6. False positive results may occur in the presence of a wide variety of traumatic and non-traumatic tumors that secrete hCG. Therefore, the possibility of an hCG secreting neoplasia should be eliminated prior to diagnosing pregnancy.
7. Any false negative results may be seen when assaysing specimens from individuals taking the drugs Pergonal® and Clomid®. Additionally Pergonal will often be followed with an injection of hCG.
8. Spontaneous microabortions and ectopic pregnancies will tend to have values which are lower than the predicted values during a normal pregnancy, while somewhat higher values are often seen in multiple pregnancies.
9. Following therapeutic abortion, detectable hCG may persist for as long as six to four weeks. The disappearance rate of hCG, after spontaneous abortion, will vary depending upon the quantity of viable residual trophoblast.
10. A hCG value alone is not of diagnostic value and should only be used in conjunction with other clinical manifestations (observations) and diagnostic procedures.

*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 population was undertaken to determine expected values for the hCG AccuBind® ELISA Test System. The mean (x) values, standard deviations (σ) and expected ranges (±2σ) are presented in Table 1.

*Table 1

<table>
<thead>
<tr>
<th>Expected Values for the hCG ELISA Test System (In mIU/ml)</th>
<th>Level 3</th>
<th>Level 2</th>
<th>Level 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>hCG (IU/ml)</td>
<td>0.5</td>
<td>1.0</td>
<td>2.5</td>
</tr>
<tr>
<td>Level 1</td>
<td>10.0</td>
<td>20.0</td>
<td>30.0</td>
</tr>
<tr>
<td>Level 2</td>
<td>18.7</td>
<td>37.5</td>
<td>56.3</td>
</tr>
<tr>
<td>Level 3</td>
<td>214.8</td>
<td>429.6</td>
<td>644.4</td>
</tr>
</tbody>
</table>

*50% Reference for hCG ELISA Test System (In mIU/ml) 50% Reference for hCG ELISA Test System

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision
The within- and between assay precisions of the hCG AccuBind® ELISA kit is determined by analyses on three different levels of control sera. The data (Table 4) are presented in Table 4 and Table 5.

*Table 5

<table>
<thead>
<tr>
<th>Table 5: Between Assay Precision* (Values in mIU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
</tr>
<tr>
<td>--------</td>
</tr>
<tr>
<td>Level 1</td>
</tr>
<tr>
<td>Level 2</td>
</tr>
<tr>
<td>Level 3</td>
</tr>
</tbody>
</table>

14.2 Sensitivity
The hCG AccuBind® ELISA test system has a sensitivity of 0.003 mIU/ml. This is equivalent to a sample containing 0.102 mIU/ml of hCG secreted by the 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

<table>
<thead>
<tr>
<th>Table 6: Correlation Coefficient (C.V.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monobind</td>
</tr>
<tr>
<td>Reference</td>
</tr>
</tbody>
</table>

Only slight differences 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 indicate excellent method agreement.

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

*Table 7

<table>
<thead>
<tr>
<th>Substance</th>
<th>Cross Reactivity</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>hCG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Folliculin (FSH)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Luteinizing Hormone (LH)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCG subunit</td>
<td>0.0001</td>
<td>1000ng/ml</td>
</tr>
<tr>
<td>Folliculin (FSH)</td>
<td>&lt; 0.0001</td>
<td>1000ng/ml</td>
</tr>
<tr>
<td>Luteinizing Hormone (LH)</td>
<td>&lt; 0.0001</td>
<td>1000ng/ml</td>
</tr>
</tbody>
</table>

15.0 REFERENCES


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