hCG + b ELISA Kit

Cat. No.:DEIA10133
Pkg.Size:96T

Intended use

The hCG + b ELISA Kit is intended for the quantitative determination of hCG/CGB in serum.

General Description

Chorionic Gonadotropin (hCG) is a glycoprotein hormone which is normally produced by the placenta during pregnancy. After conception, the hCG concentration increases rapidly to reach a peak near the end of the first trimester. High concentrations are observed throughout pregnancy. After delivery, hCG levels fall rapidly and become undetectable after a few days. Structurally intact hCG molecules are composed of an alpha and a beta subunit. The alpha subunit is nearly identical to the alpha subunits of other glycoprotein hormones, such as Thyroid Stimulating Hormone (TSH), Luteinizing Hormone (LH), and Follicle Stimulating Hormone (FSH): The differences in the beta subunit of the respective hormones account for their biological specificity and immunochemical distinctiveness. Monoclonal antibodies recognizing unique sites on the beta chain of the β-hCG/hCG molecule are essential for differentiation between hCG and LH, FSH and TSH. Specific assays for beta-hCG permit the early detection of pregnancy. In addition to the elevated hCG levels during pregnancy, high concentrations of βhCG/hCG may be associated with neoplasms of trophoblastic and nontrophoblastic origin such as hydatiform mole, chorionepithelioma, embryonal cell carcinoma, seminoma and many others.

Principle Of The Test

The HCG+beta ELISA KIT is a solid phase enzyme-linked immunosorbent assay (ELISA) based on the sandwich principle. The microtiter wells are coated with a monoclonal antibody directed towards a unique antigenic site on a β-hCG molecule. An aliquot of patient sample containing endogenous β-hCG or hCG is incubated in the coated well with enzyme conjugate, which is an anti-βhCG antiserum conjugated with horseradish peroxidase. After incubation, the unbound conjugate is washed off with water. The amount of bound peroxidase is proportional to the concentration of βhCG/hCG in the sample. Having added the substrate solution, the intensity of color developed is proportional to the concentration of βhCG/hCG in the patient sample.

Reagents And Materials Provided

1. Microtiterwells, 12 x 8 (break apart) strips, 96 wells Wells coated with anti-β-HCG monoclonal antibody.
2. Standard (Standard 0-5), 6 vials (lyoph.), 1.0 ml 0; 5; 25; 50; 100; 200 mIU/ml (1pg/ml = 0.00916 mIU/ml; 1.IRP 75/537).
3. Sample Diluent, 1 vial, 10 ml, ready to use Note: Additional Sample Diluent for Sample dilution available on request.
4. Enzyme Conjugate, 1 vial, 11 ml, ready to use Anti-HCG antiserum conjugated to horseradish peroxidase.
5. Substrate Solution , 1 vial, 11 ml, ready to use TMB.
6. Stop Solution, 1 vial, 6 ml, ready to use contains 0.5M H2SO4 Avoid contact with the stop solution. It may cause skin irritations and burns.

Materials Required But Not Supplied

1. A microtiterplate calibrated reader (450±10 nm) (e.g. the DRG Instruments Microtiterplate Reader).
2. Calibrated variable precision micropipettes (Varipette Eppendorf), Multipette Eppendorf or similar products.
3. Absorbent paper.
4. Aqua dest.
Storage

1. When stored at 2 to 8°C unopened reagents will retain reactivity until expiration date. Do not use reagents beyond this date.
2. Enzyme-Conjugate, Substrate Solution, Standards and Sample Diluent must be stored at 2 to 8°C.
3. Microtiter wells must be stored at 2 to 8°C. Once the foilbag has been open care should be taken to close it tightly again.

Specimen Collection And Handling

1. SPECIMEN COLLECTION
Collect blood by venipuncture, allow to clot, and separate serum by centrifugation at room temperature. Do not use haemolytic, icteric or lipaemic serum. ATTENTION! This kit is for use with samples without additives only.

2. SPECIMEN STORAGE
Specimens should be capped and may be stored for up to 5 days at 2-8°C prior to assaying. Specimen held for a longer time should be frozen only once at-20°C prior to assay. Thawed samples should be inverted several times prior to testing.

3. SPECIMEN DILUTION
If in an initial assay, a serum specimen is found to contain more than the highest standard, the specimens can be diluted 10-fold or 100 fold with Sample Diluent and reassayed as described in Assay Steps.
   a) dilution 1:10: 10 µl Serum + 90 µl Sample Diluent (mix thoroughly)
   b) dilution 1:100: 10 µl dilution a) 1:10 + 90 µl Sample Diluent (mix thoroughly).

Reagent Preparation

Allow all reagents and required number of strips to reach room temperature prior to use.

β-HCG Standards
Reconstitute the lyophilized contents of the standard vial with 1.0 ml Aqua dest.
Note: The reconstituted standards are stable for 2 months at 2-8°C. For longer storage freeze at-20°C.

Assay Steps

Assay Steps (quantitative method)
1. Secure the desired number of Microtiterwells in the holder.
2. Dispense 25 µl HCG Standards (0; 5; 25; 50; 100; 200 mIU/ml), controls and samples with new disposable tips into appropriate wells.
3. Dispense 100 µl Enzyme Conjugate into each well.
4. Thoroughly mix for 10 seconds. It is important to have a complete mixing in this step.
5. Incubate for 60 minutes at room temperature.
6. Briskly shake out the contents of the wells. Rinse the wells 5 times with Aqua dest. Strike the wells sharply on absorbent paper to remove residual water droplets.
Important note: The sensitivity and precision of this assay is markedly influenced by the correct performance of the washing procedure!
7. Add 100 µl of Substrate Solution to each well.
8. Incubate for 15 minutes at room temperature.
9. Stop the enzymatic reaction by adding 50 µl of Stop Solution to each well.
10. Read the OD at 450±10 nm with a microtiterplate reader within 10 minutes after adding the Stop Solution.

Assay Steps (qualitative method)
This procedure differentiates positive (pregnant) from negative samples by comparing the sample beta hCG levels with the Standards 0 and 50 mIU/ml. Patient samples are run with the Zero Standard (0 mIU/ml) and the 50 mIU/ml Standard. The Assay Steps is identical with the Quantitative Method, but step 9 and 10 is omitted.
Quality Control

It is recommended to use control samples according to state and federal regulations. The use of control samples is advised to assure the day to day validity of results. Use controls at both normal and pathological levels.

The controls and the corresponding results of the QC-Laboratory are stated in the QC certificate added to the kit. The values stated on the QC sheet always refer to the current kit lot and should be used for direct comparison of the results.

It is also recommended to make use of national or international Quality Assessment programs in order to ensure the accuracy of the results.

Employ appropriate statistical methods for analysing control values and trends. If the results of the assay do not fit to the established acceptable ranges of control materials patient results should be considered invalid.

In this case, please check the following technical areas: Pipetting and timing devices; photometer, expiration dates of reagents, storage and incubation conditions, aspiration and washing methods.

After checking the above mentioned items without finding any error contact your distributor or DRG directly.

Calculation

1. Calculate the average absorbance values for each set of standards, controls and patient samples.
2. Construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration in IU/ml with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Using the mean absorbance value for each sample determine the corresponding concentration of beta HCG in mIU/ml from the standard curve. Depending on experience and/or the availability of computer capability, other methods of data reduction may be employed.
4. Automated method: Computer programs using cubic spline, 4 PL (4 Parameter Logistics) or Logit-Log can generally give a good fit.
5. The concentration of the samples can be read directly from this standard curve. Samples with beta HCG concentration higher than that of the highest standard have to be diluted with Sample Diluent. The dilution factor has to be taken into account.

Reference Values

It is strongly recommended that each laboratory should determine its own normal and abnormal values.

The normal values and medians are summarized in the following table (all values in mIU/ml):

Table 1.

For women, pregnant in the second trimester the following MOM1 (medians) and normal values (5% Percentile and 95% Percentile) were determined: 14th week: MOM1: 30899 mIU/ml/normal values: 10303 mIU/ml-71980 mIU/ml; 15th week: MOM1: 20597 mIU/ml/normal values: 9246 mIU/ml-51666 mIU/ml; 16th week: MOM1: 14354 mIU/ml/normal values: 5266 mIU/ml-36947 mIU/ml.
mlU/ml; 17h week: median: 10965 mlU/ml/normal values: 4632 mlU/ml-24033 mlU/ml. Reference: 1 IRP 75/537 (WHO)

**CAUTION:**
1. Samples with expected values greater 200 mlU/ml should be diluted with Sample Diluent before assaying.
2. For the detection of pregnancy in serum, a qualitative assay is used with a cut-off point of 50 mlU/ml.
3. Negative or borderline results should be repeated on a fresh specimen obtained at least 48 hours after the first specimen.
4. It has been shown that immunological pregnancy tests may yield false results in cases of several medications and diseases such as rheumatoid arthritis. In such cases, the interpretation of the pregnancy test should be done carefully.

**Interpretation of Results**

For a qualitative analysis of the hCG level the color development of the specimen is compared with the color of the 0 and 50 mlU/ml reference standards.

If the blue color is less intense than the color of the 50 mlU/ml reference standard, the sample is considered as negative.

If the blue color is more intense than or equal to the color of the 50 mlU/ml reference standard the sample is considered as positive.

Below is listed a typical example of a standard curve with the HCG+beta ELISA.

**Table 2.**

<table>
<thead>
<tr>
<th>Standard</th>
<th>Optical Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard 0 (0 mlU/ml)</td>
<td>0.04</td>
</tr>
<tr>
<td>Standard 1 (5 mlU/ml)</td>
<td>0.15</td>
</tr>
<tr>
<td>Standard 2 (25 mlU/ml)</td>
<td>0.28</td>
</tr>
<tr>
<td>Standard 3 (50 mlU/ml)</td>
<td>0.53</td>
</tr>
<tr>
<td>Standard 4 (100 mlU/ml)</td>
<td>0.94</td>
</tr>
<tr>
<td>Standard 5 (200 mlU/ml)</td>
<td>1.50</td>
</tr>
</tbody>
</table>

**Sensitivity**

The minimum detectable concentration of beta HCG by this assay is estimated to be < 1mlU/ml.

**Specificity**

The following hormones were tested for cross-reactivity of the assay:

**Table 3.**
### Linearity

**Linearity:**

*Table 4.*

<table>
<thead>
<tr>
<th>Sample</th>
<th>Dilution</th>
<th>Mean Conc. (U/ml)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>undiluted</td>
<td>109.8</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>1:2</td>
<td>56.6</td>
<td>103</td>
</tr>
<tr>
<td></td>
<td>1:4</td>
<td>28.2</td>
<td>103</td>
</tr>
<tr>
<td></td>
<td>1:8</td>
<td>14.4</td>
<td>105</td>
</tr>
<tr>
<td></td>
<td>1:16</td>
<td>7.41</td>
<td>108</td>
</tr>
<tr>
<td>2</td>
<td>undiluted</td>
<td>117</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>1:2</td>
<td>54.7</td>
<td>94</td>
</tr>
<tr>
<td></td>
<td>1:4</td>
<td>30.1</td>
<td>103</td>
</tr>
<tr>
<td></td>
<td>1:8</td>
<td>15.8</td>
<td>108</td>
</tr>
<tr>
<td></td>
<td>1:16</td>
<td>7.69</td>
<td>105</td>
</tr>
<tr>
<td>3</td>
<td>undiluted</td>
<td>94.9</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>1:2</td>
<td>42.9</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>1:4</td>
<td>25</td>
<td>105</td>
</tr>
<tr>
<td></td>
<td>1:8</td>
<td>12.8</td>
<td>108</td>
</tr>
<tr>
<td></td>
<td>1:16</td>
<td>6.5</td>
<td>110</td>
</tr>
</tbody>
</table>

### Recovery

**Recovery:**

*Table 5.*
Reproducibility

Intra Assay Variation:
The within assay variability is shown below:
Table 6.

<table>
<thead>
<tr>
<th>Sample</th>
<th>n</th>
<th>Mean (IU/ml)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20</td>
<td>5.9</td>
<td>8.9</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>18.5</td>
<td>4.0</td>
</tr>
<tr>
<td>3</td>
<td>20</td>
<td>148.1</td>
<td>3.4</td>
</tr>
</tbody>
</table>

Inter Assay Variation:
The between assay variability is shown below:
Table 7.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean (IU/ml)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6</td>
<td>9.9</td>
</tr>
<tr>
<td>2</td>
<td>17</td>
<td>7.3</td>
</tr>
<tr>
<td>3</td>
<td>140</td>
<td>6.6</td>
</tr>
</tbody>
</table>

Precautions

1. For information on hazardous substances included in the kit please refer to Material Safety Data Sheets.
2. All reagents of this test kit which contain human serum or plasma have been tested and confirmed negative for HIV I/II,
HBsAg and HCV by FDA approved procedures. All reagents, however, should be treated as potential biohazards in use and for disposal.

3. Avoid contact with Stop Solution containing 0.5 M H2SO4. It may cause skin irritation and burns.

4. Never pipet by mouth and avoid contact of reagents and specimens with skin and mucous membranes.

5. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.

6. Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of reagents or specimens may give false results.

7. Handling should be in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.

8. Do not use reagents beyond expiry date as shown on the kit labels.

9. All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes.

10. Do not mix or use components from kits with different lot numbers. It is advised not to exchange wells of different plates even if the same lot. The kits may have been shipped or stored under different conditions and the binding characteristics of the plates may result slightly different.

11. Chemicals and prepared or used reagents have to be treated as hazardous waste according to the national biohazard safety guideline or regulation.

12. Safety Data Sheets for this product are available upon request directly from DRG Instruments GmbH. The Safety Data Sheets fit the demands of: EU-Guideline 91/155 EC.

13. In case of any severe damage of the test kit or components, DRG have to be informed written, latest one week after receiving the kit. Severely damaged single components should not be used for a test run. They have to be stored until a final solution has been found. After this, they should be disposed according to the official regulations.

14. All standards, samples, and controls should be run in duplicate concurrently so that all conditions of testing are the same.

15. The concentration of the samples can be read directly from this standard curve. Samples with a concentration higher than that of the highest standard have to be diluted with Sample Diluent. For the calculation of the concentrations this dilution factor has to be taken into account. A gradual 1:100 dilution is recommended: Dilute the patient serum 1:10 with Sample Diluent, then dilute this solution once again 1:10 with Sample Diluent.

16. All reagents and specimens must be allowed to come to room temperature before use. All reagents must be mixed without foaming.

17. Once the test has been started, all steps should be completed without interruption.

18. Use new disposal plastic pipet tips for each standard, control of sample in order to avoid cross contamination

19. Absorbance is a function of the incubation time and temperature. Before starting the assay, it is recommended that all reagents be ready, caps removed, all needed wells secured in holder, etc. This will ensure equal elapsed time for each pipetting step without interruption.

20. The present kit is adjusted to give an absorption for standard 5 > 1.200 within 10 minutes at room temperature. As a general rule the enzymatic reaction is linearly proportional to time and temperature. Therefore, if the Optical Density is too high or too low, the substrate incubation time can be decreased or increased, respectively.

21. RELIABILITY OF RESULTS: The test must be performed exactly as per the manufacturer’s instructions for use. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable national standards and/or laws. This is especially relevant for the use of control reagents. It is important to always include, within the test procedure, a sufficient number of controls for validating the accuracy and precision of the test. The test results are valid only if all controls are within the specified ranges and if all other test parameters are also within the given assay specifications. In case of any doubt or concern please contact DRG.

22. THERAPEUTICAL CONSEQUENCES: Therapeutical consequences should never be based on laboratory results alone even if all test results are in agreement with the items as stated under point 21. Any laboratory result is only a part of the total clinical picture of a patient. Only in cases where the laboratory results are in acceptable agreement with the overall clinical
picture of the patient should therapeutical consequences be derived. The test result itself should never be the sole determinant for deriving any therapeutical consequences.

23. LIABILITY: Any modification of the test kit and/or exchange or mixture of any components of different lots from one test kit to another could negatively affect the intended results and validity of the overall test. Such modification and/or exchanges invalidate any claim for replacement. Claims submitted due to customer misinterpretation of laboratory results subject to point 22 are also invalid. Regardless, in the event of any claim, the manufacturer's liability is not to exceed the value of the test kit. Any damage caused to the test kit during transportation is not subject to the liability of the manufacturer.

**Limitations**

1. INTERFERING SUBSTANCES
Any improper handling of samples or modification of this test might influence the results. Interferences caused by improper sample handling are explained in the chapters 'Specimen-Collection'.
Haemoglobin (up to 4 mg/ml), Bilirubin (up to 0.5 mg/ml) and Triglyceride (up to 30 mg/ml) have no influence on the assay results.

2. HIGH-DOSE-HOOK EFFECT
No hook effect was observed in this test up to 158,600 IU/ml of beta HCG.

**REFERENCES**