Instructions for Use

An immunoenzymatic assay for the quantitative measurement of Chorionic Gonadotropin beta in Human Serum.

This product is for research use only and is not intended for diagnostic use.
Table of Contents

INTRODUCTION
1. BACKGROUND 2
2. ASSAY SUMMARY 3
3. PRECAUTIONS 4

GENERAL INFORMATION
4. STORAGE AND STABILITY 4
5. MATERIALS SUPPLIED 4
6. MATERIALS REQUIRED, NOT SUPPLIED 5
7. LIMITATIONS 6
8. TECHNICAL HINTS 7

ASSAY PREPARATION
9. REAGENT PREPARATION 8
10. SAMPLE COLLECTION AND STORAGE 8
11. PLATE PREPARATION 9

ASSAY PROCEDURE
12. ASSAY PROCEDURE 10

DATA ANALYSIS
13. CALCULATIONS 12
14. TYPICAL DATA 13
15. TYPICAL SAMPLE VALUES 14
16. ASSAY SPECIFICITY 15

RESOURCES
17. TROUBLESHOOTING 16
18. NOTES 18
INTRODUCTION

1. BACKGROUND

Abcam’s Chorionic Gonadotropin beta in vitro ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for the accurate quantitative measurement of Chorionic Gonadotropin beta in Human serum. A 96-well plate has been precoated with anti-Chorionic Gonadotropin beta antibodies. Samples and standards and are added to the wells, where Chorionic Gonadotropin beta in the sample and standards binds to the precoated antibody. After incubation and washing, added anti-Chorionic Gonadotropin beta HRP conjugate binds to this antibody-Chorionic Gonadotropin beta complex. After incubation, the wells are washed to remove unbound material and TMB substrate is then added which is catalyzed by HRP to produce blue coloration. The reaction is terminated by addition of Stop Solution which stops the color development and produces a color change from blue to yellow. The intensity of signal is directly proportional to the amount of Chorionic Gonadotropin beta in the sample and the intensity is measured at 450 nm.

Human Chorionic Gonadotropin (hCG) is a glycoprotein hormone normally produced by placenta during pregnancy. The hormone is present in blood and urine around seven to thirteen days following implantation of the fertilized ovum. Structurally intact hCG molecules consist of two non-covalently linked polypeptide subunits, the alpha and beta chain subunits. Measurement of intact hCG and of the alpha subunit of hCG appears to give similar results in blood and urine but not the levels of beta subunit. In the normal second-trimester maternal sera, the level of intact hCG range from 20,000 mU/mL to 50,000 mU/mL (1 ng = 15 mU). In contrast, the levels of either free α- or free β-hCG are on average one half of 1% of hCG levels. hCG and the free subunits appear not to be useful as serological markers for nontrophoblastic tumors; however, the absolute increase of β-hCG level in choriocarcinoma patients clearly differentiates it from normal pregnancy.
2. **ASSAY SUMMARY**

Prepare all reagents, samples and standards as instructed.

Add samples and standards to wells used. Add Zero Buffer and mix well. Incubate at room temperature.

After washing, add prepared labeled HRP-Conjugate to each well. Incubate at room temperature.

After washing, add TMB substrate solution to each well. Incubate at room temperature. Add Stop Solution to each well. Read immediately.
GENERAL INFORMATION

3. PRECAUTIONS
Please read these instructions carefully prior to beginning the assay.
All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY
Store kit at 2-8°C immediately upon receipt.
Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in section 9. Reagent Preparation.

5. MATERIALS SUPPLIED

<table>
<thead>
<tr>
<th>Item</th>
<th>Amount</th>
<th>Storage Condition (Before Preparation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-Chorionic Gonadotropin beta Coated Microplate (12 x 8 wells)</td>
<td>96 Wells</td>
<td>2-8°C</td>
</tr>
<tr>
<td>Stop Solution</td>
<td>11 mL</td>
<td>2-8°C</td>
</tr>
<tr>
<td>Anti-Chorionic Gonadotropin beta HRP Conjugate</td>
<td>18 mL</td>
<td>2-8°C</td>
</tr>
<tr>
<td>TMB Substrate Solution</td>
<td>11 mL</td>
<td>2-8°C</td>
</tr>
<tr>
<td>Zero Buffer</td>
<td>13 mL</td>
<td>2-8°C</td>
</tr>
<tr>
<td>Chorionic Gonadotropin beta Standard 1 – 0 ng/mL (Lyophilized)</td>
<td>1 vial</td>
<td>2-8°C</td>
</tr>
<tr>
<td>Chorionic Gonadotropin beta Standard 2 – 2.5 ng/mL (Lyophilized)</td>
<td>1 vial</td>
<td>2-8°C</td>
</tr>
<tr>
<td>Chorionic Gonadotropin beta Standard 3 – 5 ng/mL (Lyophilized)</td>
<td>1 vial</td>
<td>2-8°C</td>
</tr>
<tr>
<td>Chorionic Gonadotropin beta Standard 4 – 10 ng/mL (Lyophilized)</td>
<td>1 vial</td>
<td>2-8°C</td>
</tr>
<tr>
<td>Chorionic Gonadotropin beta Standard 5 – 25 ng/mL (Lyophilized)</td>
<td>1 vial</td>
<td>2-8°C</td>
</tr>
<tr>
<td>Chorionic Gonadotropin beta Standard 6 – 50 ng/mL (Lyophilized)</td>
<td>1 vial</td>
<td>2-8°C</td>
</tr>
</tbody>
</table>
6. **MATERIALS REQUIRED, NOT SUPPLIED**

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Microplate reader capable of measuring absorbance at 450 nm or 620 nm
- Incubator at 37°C
- Multi- and single-channel pipettes to deliver volumes between 10 and 1,000 µL
- Optional: Automatic plate washer for rinsing wells.
- Rotating mixer
- Deionised or (freshly) distilled water.
- Disposable tubes
- Timer
- Absorbent paper or paper towel.
7. **LIMITATIONS**

- ELISA kit intended for research use only. Not for use in diagnostic procedures

- All components of Human origin used for the production of these reagents have been tested for **anti-HIV antibodies**, **anti-HCV antibodies** and **HBsAg** and have been found to be non-reactive. Nevertheless, all materials should still be regarded and handled as potentially infectious

- Use only clean pipette tips, dispensers, and lab ware

- Do not interchange screw caps of reagent vials to avoid cross-contamination

- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination

- After first opening and subsequent storage check conjugate and control vials for microbial contamination prior to further use

- To avoid cross-contamination and falsely elevated results pipette patient samples and dispense conjugate, without splashing, accurately to the bottom of wells

- Serum samples demonstrating gross lipemia, gross hemolysis, or turbidity should not be used with this test.
8. **TECHNICAL HINTS**

- Avoid foaming or bubbles when mixing or reconstituting components
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions
- Ensure plates are properly sealed or covered during incubation steps
- Complete removal of all solutions and buffers during wash steps is necessary for accurate measurement readings
- Addition of the TMB Substrate solution initiates a kinetic reaction, which is terminated by the addition of the Stop Solution. Therefore, the TMB Substrate and the Stop Solution should be added in the same sequence to eliminate any time deviation during the reaction
- It is important that the time of reaction in each well is held constant for reproducible results. Pipetting of samples should not extend beyond ten minutes to avoid assay drift. If more than 10 minutes are needed, follow the same order of dispensation. If more than one plate is used, it is recommended to repeat the dose response curve in each plate
- The incomplete or inaccurate liquid removal from the wells could influence the assay precision and/or increase the background
- **This kit is sold based on number of tests.** A ‘test’ simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions
9. **REAGENT PREPARATION**

Equilibrate all reagents, samples and controls to room temperature (18-25°C) prior to use.

9.1 **Chorionic Gonadotropin beta Standards**

Reconstitute each lyophilized Chorionic Gonadotropin beta Standard with 1.0 mL distilled water. Allow the reconstituted material to stand for at least 20 minutes and mix gently. Reconstituted standards will be stable for up to 30 days when stored sealed at 2-8°C.

- All other solutions are supplied ready to use

10. **SAMPLE COLLECTION AND STORAGE**

- Serum should be prepared from a whole blood specimen obtained by acceptable medical techniques. This kit is for use with serum samples without additives only.

- The determination of Chorionic Gonadotropin beta can be performed in Human serum. Microbiologically contaminated, highly lipemic or haemolysed should not be used in the assay. If the assay is performed on the same day of sample collection, the specimen should be kept at 2-8°C; otherwise it should be aliquoted and stored deep-frozen (-20°C). If samples are stored frozen, mix thawed samples gently for 5 minutes before testing.

  *Avoid repeated freezing and thawing*
11. **PLATE PREPARATION**

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
- Unused well strips should be returned to the plate packet and stored at 4°C.
- For each assay performed, a minimum of 1 well must be used as a blank, omitting sample and conjugate from well addition.
- For statistical reasons, we recommend each standard and sample should be assayed with a minimum of two replicates (duplicates).
12. ASSAY PROCEDURE

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- Please read the test protocol carefully before performing the assay. Result reliability depends on strict adherence to the test protocol as described.
- If performing the test on ELISA automatic systems we recommend increasing the washing steps from three to five and the volume of washing solution from 300 µL to 350 µL to avoid washing effects.
- Assay all standards, controls and samples in duplicate.
  13.1. Prepare all reagents, working standards, and samples as directed in the previous sections.
  13.2. Add 50 µL standards, control and samples into their respective wells.
  13.3. Add 100 µL of Zero Buffer into each well. Mix gently for 30 seconds.
      Note: Complete mixing is essential for good assay performance
  13.4. Cover wells with the foil supplied in the kit and incubate at room temperature for 30 minutes.
  13.5. Remove the foil, aspirate the contents of the wells and wash each well five times with 300 µL of deionized or distilled water. Avoid spill over into neighboring wells. The soak time between each wash cycle should be >5 sec. After the last wash, remove the remaining deionized or distilled water by aspiration or decanting. Invert the plate and blot it against clean paper towels to remove excess liquid.
      Note: Complete removal of liquid at each step is essential for good assay performance.
  13.6. Add 150 µL of Anti-Chorionic Gonadotropin beta HRP Conjugate into each well. Gently mix for 10 seconds.
  13.7. Incubate at room temperature for 30 minutes.
13.8. Repeat wash step 13.6
13.9. Add 100 µL TMB Reagent into each well. Gentle mix for 10 seconds.
13.10. Incubate at room temperature for 20 minutes in the dark.
13.11. Stop the reaction by adding 100 µL of Stop Solution to each well. Mix gently for 30 seconds.
   Note: It is important to make sure that all the blue color changes to yellow color completely.
13.12. Measure the absorbance of the sample at 450 nm within 15 minutes of addition of the Stop Solution.
13. **CALCULATIONS**

Calculate the mean background subtracted absorbance for each point of the standard curve and each sample. Plot the mean value of absorbance of the standards against concentration. Draw the best-fit curve through the plotted points. (e.g.: Four Parameter Logistic).

Interpolate the values of the samples on the standard curve to obtain the corresponding values of the concentrations expressed in ng/mL.
14. **TYPICAL DATA**

**TYPICAL STANDARD CURVE** – Data provided for demonstration purposes only. A new standard curve must be generated for each assay performed.

<table>
<thead>
<tr>
<th>Conc. (ng/mL)</th>
<th>Mean O.D. (-Blank)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.061</td>
</tr>
<tr>
<td>2.5</td>
<td>0.296</td>
</tr>
<tr>
<td>5.0</td>
<td>0.498</td>
</tr>
<tr>
<td>10.0</td>
<td>0.929</td>
</tr>
<tr>
<td>25.0</td>
<td>1.711</td>
</tr>
<tr>
<td>50.0</td>
<td>2.613</td>
</tr>
</tbody>
</table>
15. **TYPICAL SAMPLE VALUES**

**REFERENCE VALUES –**

**hCG and Free β-hCG Subunit Levels in Normal Pregnancy**

A logarithmic increase in the serum concentration of hCG was observed from 5-8 weeks of gestation (2,600 ng/mL to 33,000 ng/mL) as defined by last menstrual period; thereafter, hCG values decreased. Similarly, free β-hCG levels increased rapidly to reach maximum levels (~60 ng/ml) at 8-9 weeks of pregnancy, followed by a gradual decline during the next 11-12 weeks of gestation.

At 5 weeks of gestation, the ratio of free β-hCG to intact hCG is approximately 1.0 % (w/w). Thereafter, this ratio remains remarkably constant over 22 weeks of gestation (~ 0.5 % w/w).

**hCG and Free Subunits Levels in Gestational Choriocarcinoma**

Free α and free β-subunits and hCG levels were measured in five patients with untreated gestational choriocarcinoma. The concentrations in serum are shown in the following table:

<table>
<thead>
<tr>
<th>Patient Number</th>
<th>hCG (ng/mL)</th>
<th>α-hCG (ng/mL)</th>
<th>β-hCG (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>210,000</td>
<td>112</td>
<td>8,000</td>
</tr>
<tr>
<td>2</td>
<td>22,195</td>
<td>20</td>
<td>1,300</td>
</tr>
<tr>
<td>3</td>
<td>6,840</td>
<td>1</td>
<td>232</td>
</tr>
<tr>
<td>4</td>
<td>36,000</td>
<td>44</td>
<td>3,900</td>
</tr>
<tr>
<td>5</td>
<td>4,200</td>
<td>2</td>
<td>350</td>
</tr>
</tbody>
</table>

The levels of free α-hCG were low, ranging from 1-112 ng/mL, whereas hCG levels ranged from 4,200 to 210,000 ng/mL (1 ng ≈ 15 mU). In contrast, free β-hCG concentrations were found to be markedly elevated in choriocarcinoma.
DATA ANALYSIS

SENSITIVITY –
The minimum detectable concentration of Chorionic Gonadotropin beta is estimated to be 0.25 ng/mL.

16. ASSAY SPECIFICITY
This kit detects Chorionic Gonadotropin beta in Human samples. Other species have not yet been tested with this kit.
## 17. TROUBLESHOOTING

<table>
<thead>
<tr>
<th>Problem</th>
<th>Cause</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low signal</td>
<td>Incubation time too short</td>
<td>Try overnight incubation at 4 °C</td>
</tr>
<tr>
<td></td>
<td>Precipitate can form in wells upon substrate addition when concentration of target is too high</td>
<td>Increase dilution factor of sample</td>
</tr>
<tr>
<td></td>
<td>Using incompatible sample type (e.g. serum vs. cell extract)</td>
<td>Detection may be reduced or absent in untested sample types</td>
</tr>
<tr>
<td></td>
<td>Sample prepared incorrectly</td>
<td>Ensure proper sample preparation/dilution</td>
</tr>
<tr>
<td>Large CV</td>
<td>Bubbles in wells</td>
<td>Ensure no bubbles present prior to reading plate</td>
</tr>
<tr>
<td></td>
<td>All wells not washed equally/thoroughly</td>
<td>Check that all ports of plate washer are unobstructed/wash wells as recommended</td>
</tr>
<tr>
<td></td>
<td>Incomplete reagent mixing</td>
<td>Ensure all reagents/master mixes are mixed thoroughly</td>
</tr>
<tr>
<td></td>
<td>Inconsistent pipetting</td>
<td>Use calibrated pipettes &amp; ensure accurate pipetting</td>
</tr>
<tr>
<td></td>
<td>Inconsistent sample preparation or storage</td>
<td>Ensure consistent sample preparation and optimal sample storage conditions (e.g. minimize freeze/thaws cycles)</td>
</tr>
<tr>
<td>Problem</td>
<td>Cause</td>
<td>Solution</td>
</tr>
<tr>
<td>-------------------------</td>
<td>------------------------------------</td>
<td>---------------------------------------------------</td>
</tr>
<tr>
<td>Wells are insufficiently washed</td>
<td>Wash wells as per protocol recommendations</td>
<td></td>
</tr>
<tr>
<td>Contaminated wash buffer</td>
<td>Make fresh wash buffer</td>
<td></td>
</tr>
<tr>
<td>Waiting too long to read plate after adding stop solution</td>
<td>Read plate immediately after adding stop solution</td>
<td></td>
</tr>
<tr>
<td>Improper storage of ELISA kit</td>
<td>Store all reagents as recommended. Please note all reagents may not have identical storage requirements.</td>
<td></td>
</tr>
<tr>
<td>Using incompatible sample type (e.g. Serum vs. cell extract)</td>
<td>Detection may be reduced or absent in untested sample types</td>
<td></td>
</tr>
</tbody>
</table>
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