Instructions for Use

For the qualitative measurement of Human Chorionic Gonadotropin concentrations in serum and urine

This product is for research use only and is not intended for diagnostic use.
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1. Introduction

ab108637, Chorionic Gonadotropin Human ELISA Kit is intended for the qualitative determination of Human Chorionic Gonadotropin (hCG) in human urine or serum.

Human chorionic gonadotropin (hCG) is a glycoprotein hormone secreted by the developing placenta shortly after fertilization. In normal pregnancy, hCG can be detected generally as early as 7 days following conception, doubling every 1.3 to 2 days. At the time of the first missed menstrual period, hCG concentration is about 100 mIU/ml, and peak levels of 100,000-200,000 mIU/ml are seen at the end of the first trimester. The appearance of hCG soon after conception and its subsequent rise in concentration during early gestational growth make it an excellent marker for the early detection of pregnancy.
2. Assay Summary

ab108637 is a sandwich enzyme immunoassay for the determination of Human Chorionic Gonadotropin in urine or serum.

The method employs two monoclonal antibodies to selectively identify hCG in urine/serum with a high degree of sensitivity. In less than 10 minutes, elevated levels of hCG as little as 20 mIU/ml can be detected.

The test sample is allowed to react with the antibody enzyme conjugate and the antibodies on the solid phase simultaneously.

In the presence of hCG, a specific antibody-hCG-antibody-enzyme complex will form on the surface of microtiter well.

After unbound enzyme conjugate is removed by rinsing under a stream of distilled water, the well is incubated with TMB Reagent. The development of blue color in the well indicated the presence of hCG.

Comparing the color intensity of patient samples with that of the provided known reference, the amount of hCG can be visually estimated to be greater or less than 20 mIU/ml.
3. Kit Contents

- Microtiter Wells: mouse monoclonal anti-β-hCG coated wells, 96 wells.
- Enzyme Conjugate: containing mouse monoclonal anti-β-hCG peroxidase in protein stabilizer, 7 ml (Red cap).
- HCG Standard: contains 0 mIU/ml hCG, 1 ml (White cap).
- HCG Standard: contains 20 mIU/ml hCG, 1 ml (Yellow cap).
- HCG Standard: contains 150 mIU/ml hCG, 1 ml (Black cap).
- TMB Reagent (One-Step), 7 ml (Amber cap).
- Stop Solution: 1N HCl, 7 ml (Natural cap).

4. Storage and Handling

Store reagents at refrigerator temperature (2-8°C) when not in use. Do not freeze. Bring reagents and specimens to room temperature (18-25°C) before testing.
5. Additional Materials Required

- Specimen collection containers
- Timer
- Distilled or deionized water
- Absorbent paper towels

6. Preparation of Reagents

All reagents should be allowed to reach room temperature (18-25°C) before use.

7. Preparation and Collection of Specimen

1. The specimen must be collected in a clean, dry container, either plastic or glass, without preservative. Specimen collected at anytime may be used. However, the first morning urine generally contains the highest concentration of hCG.

2. All specimens may be refrigerated (2-8°C) and stored up to 72 hours prior to testing. If specimens are refrigerated, they must be equilibrated to room temperature before testing. Urine specimens exhibiting visible precipitates should be filtered, centrifuged or allowed to settle.
3. No special preparation of the patient specimen is required. Additives such as sodium azide should be avoided. Limited sample studies indicated that plasma sample prepared from EDTA can be used in lieu of serum. Serum not to be assayed immediately must be stored in a refrigerator or a freezer. Bring these specimens to room temperature prior to testing. Do not freeze and thaw repeatedly.

8. Assay Method

Qualitative ELISA Testing:

1. Place Microtiter Wells for your test on the holder.
2. Dispense 1 drop (50 µl) of test sample and/or 1 drop (50 µl) of hCG Standards and Negative Reference, if desired, into the appropriately labeled Microtiter Wells. Use a separate disposable pipette for each sample.
3. Add 1 drop (50 µl) of Enzyme Conjugate into each well. Mix gently for 10 seconds.
4. Incubate at room temperature (18-25°C) for 5 minutes.
5. Remove content by flicking the microtiter well holder into sink, followed by rinsing the wells 5 times with distilled or deionized water. Note: Avoid well to well contamination from water overflow during the first rinse. Separating wells on the well holder would help.
6. Add 1 drop of TMB Reagent into each well. Mix gently for 10 seconds.
7. Incubate at room temperature (18-25°C) for 5 minutes.
8. Compare the color developed in specimen wells to that of the positive reference well (20 mIU/ml).

Quantitative Reader Procedure:

1. In order to run a standard curve, test hCG standards included in the kit by the same method as the test samples.
2. Following step 7 above, if a microtiter reader is available for quantitative reading at 450 nm, rapidly add 1 drop (50 µl) of Stop Solution (1N HCl) into each well including your test specimen, all hCG Standards and Negative Reference.
3. Read absorbance at 450 nm with a microtiter well reader within 15 minutes.
4. Calculate the hCG concentration from the standard curve.
9. Data Analysis

1. **Positive**: Wells showing blue color stronger than the 20 mIU/ml Reference Standard indicate the presence of hCG, or positive results.

2. **Negative**: Wells showing no color or faint blue color weaker than the 20 mIU/ml Reference Standard indicate non-detectable amount or less than 20 mIU/ml of hCG in the specimen.

   A slight bluish tinge, much lighter than the positive reference well, may result from insufficient washing and should be considered negative. If the patient sample shows a negative result but pregnancy is suspected, the test should be repeated using a fresh specimen obtained 2-3 days later.

3. **Note:**
   
   a. Depending on the concentration of hCG in the specimen, the color may develop instantaneously.

   b. Incubation of the TMB Reagent beyond 5 minutes may result in a slight shade of blue much less intense than that of the positive reference (20 mIU/ml). This should still be considered as negative.
A. Sensitivity

The sensitivity of ab108637 is set at 20 mIU/ml. The 20 mIU/ml Positive reference (calibrated to the 2nd international Standard) was designed as the cut off for the test because hCG concentrations in this range are usually achieved during the 2nd week after conception.

B. Accuracy

ab108637 shows 99.4% agreement with results obtained by the use of other qualified immunological pregnancy tests under actual clinical conditions.

Urine samples from five known non-pregnant subjects were spiked with hCG to concentrations of 0, 40, 100 mIU/ml. A total of 75 of these samples were blind labeled and tested with ab108637. Results are summarized below.

<table>
<thead>
<tr>
<th>HCG (mIU/ml)</th>
<th>0</th>
<th>40</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of samples</td>
<td>25</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>Negative</td>
<td>25</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Positive</td>
<td>0</td>
<td>25</td>
<td>25</td>
</tr>
</tbody>
</table>
10. Limitations

- Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the package insert instructions and with adherence to good laboratory practice.
- The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
- Serum samples demonstrating gross lipemia, gross hemolysis, or turbidity should not be used with this test.
11. Specificity

Specificity of ab108637 was determined from cross reaction studies with known amounts of Luteinizing Hormone (LH), Follicle Stimulating Hormone (FSH), and Thyroid Stimulating Hormone (TSH). 500 mIU/ml LH, 1000 mIU/ml FSH and 1000 µIU/ml TSH all give negative results to the test.

The following substances were added in 20 mIU/ml hCG spiked negative urine samples. None of the substances at concentration tested interfered in the assay.

<table>
<thead>
<tr>
<th>Material Tested</th>
<th>Test Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetaminophen</td>
<td>20 mg/dl</td>
</tr>
<tr>
<td>Acetylsalicylic Acid</td>
<td>20 mg/dl</td>
</tr>
<tr>
<td>Ascorbic Acid</td>
<td>20 mg/dl</td>
</tr>
<tr>
<td>Atropine</td>
<td>20 mg/dl</td>
</tr>
<tr>
<td>Caffeine</td>
<td>20 mg/dl</td>
</tr>
<tr>
<td>Gentisic Acid</td>
<td>20 mg/dl</td>
</tr>
<tr>
<td>Glucose</td>
<td>2 g/dl</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>1 mg/dl</td>
</tr>
</tbody>
</table>
# 12. Troubleshooting

<table>
<thead>
<tr>
<th>Problem</th>
<th>Cause</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poor standard curve</td>
<td>Improper standard dilution</td>
<td>Confirm dilutions made correctly</td>
</tr>
<tr>
<td></td>
<td>Standard improperly reconstituted (if applicable)</td>
<td>Briefly spin vial before opening; thoroughly resuspend powder (if applicable)</td>
</tr>
<tr>
<td></td>
<td>Standard degraded</td>
<td>Store sample as recommended</td>
</tr>
<tr>
<td></td>
<td>Curve doesn't fit scale</td>
<td>Try plotting using different scale</td>
</tr>
<tr>
<td>Low signal</td>
<td>Incubation time too short</td>
<td>Try overnight incubation at 4 °C</td>
</tr>
<tr>
<td></td>
<td>Target present below detection limits of assay</td>
<td>Decrease dilution factor; concentrate samples</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>High background</td>
<td>Wells are insufficienly washed</td>
<td>Wash wells as per protocol recommendations</td>
</tr>
<tr>
<td></td>
<td>Contaminated wash buffer</td>
<td>Make fresh wash buffer</td>
</tr>
<tr>
<td></td>
<td>Waiting too long to read plate after adding STOP solution</td>
<td>Read plate immediately after adding STOP solution</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>-----------------</td>
<td>-------------------------------------------------</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 and 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>Low sensitivity</td>
<td>Improper storage of ELISA kit</td>
<td>Store all reagents as recommended. Please note all reagents may not have identical storage requirements.</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>
</tbody>
</table>

For further technical questions please do not hesitate to contact us by email ([technical@abcam.com](mailto:technical@abcam.com)) or phone (select “contact us” on [www.abcam.com](http://www.abcam.com) for the phone number for your region).
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