i-CHROMA™ hCG

ImmuNoAssay for Quantitative Measurement of hCG in Human Serum / Plasma with i-CHROMA™ Reader System

INTENDED USE
i-CHROMA hCG Test along with the i-CHROMA™ Reader is a fluorescence immunoassay that quantifies human chorionic gonadotrophin(hCG) concentration in serum/plasma. The test is used as an aid in the early detection of pregnancy.

INTRODUCTION
Human chorionic gonadotropin (hCG) is a glycoprotein hormone secreted by the developing placenta shortly after implantation. hCG can be detected in the urine and serum of pregnant women as early as 6 to 15 days after conception. The concentration of hCG increases to 50 mIU/ml one week post implantation and reaches to about 100 mIU/ml at the first missed menstrual period and the peak at 100,000-200,000 mIU/ml at the the first trimester. The i-CHROMA™ hCG Test measures quantitatively hCG concentration in human serum/plasma.

PRINCIPLE
The test uses a sandwich immunodetection method, such that the detector antibody in buffer binds to hCG in blood sample and antigen-antibody complexes are captured to antibody that has been immobilized on test strip as sample mixture migrates nitrocellulose matrix. Thus the more hCG antigen in blood, the more antigen-antibody complexes accumulated on test strip. Signal intensity of fluorescence on detector antibody reflects amount of antigen captured and is processed from i-CHROMA™ Reader to show hCG concentration in specimen. The working range of i-CHROMA™ hCG test is 5-10,000 mIU/ml.

COMPOSITION OF REAGENTS
The i-CHROMA™ hCG consists of Test Device, ID Chip, and Detector Buffer. Test Device is individually sealed with a desiccant in aluminum pouch, and Detector Buffer is dispensed individually in a tube. A pouch containing the predispensed tubes is delivered separately from Test Device in a Styrofoam box filled with ice pack.

- Test Device contains a test strip in which mouse anti-hCG antibody and streptavidin have been immobilized on the test and on the control line of strip, respectively.
- Detector Buffer, predispensed in a tube, contains fluorescence-labeled anti-human hCG antibody, fluorescence-biotin labeled BSA, gelatin as a stabilizer, and sodium azide as a preservative in PBS.

WARNINGS AND PRECAUTIONS
- IVD For In Vitro Diagnostic Use.
- Carefully follow the instructions and procedures described in this insert. REF Catalog No. 13009
- Don’t use Test Device if its lot number does not match with EEPROM card number that is inserted onto the instrument.
- The i-CHROMA™ hCG is only operational in the i-CHROMA™ Reader. And tests should be applied by trained staff working in the laboratories where the sample(s) is taken by qualified medical personnel.
- LOT Neither inter-change materials from different product lots nor use beyond the expiration date. The use of medical device beyond expiration date may affect on test result.
- The i-CHROMA™ hCG Device should remain in its original sealed pouch until ready to use. Do not use the Test Device if the pouch is damaged or the seal is broken. Discard after single use.
- The i-CHROMA™ hCG Device and Reader should be used away from vibration and magnetic field. During normal usage, i-CHROMA™ hCG may introduce minute vibration, which should be regarded normal.
- Use separate clean pipette tips and sample vials for different specimens. The pipette tips and sample vials should be used or one specimen only. Discard after single use.
- Blood specimens, used test devices, pipette tips and sample vials are potentially infectious. Proper laboratory safety techniques, handling and disposal methods should be followed in accordance with standard procedures and relevant regulations observed by microbiological hazard materials.
- The i-CHROMA™ hCG provides a presumptive diagnosis for pregnancy. A confirmed pregnancy diagnosis should only be made by a physician after all clinical and laboratory findings have been evaluated.
- The test will be applied on a routine basis and not in emergency situations.
- Do not smoke, eat, or drink in areas in which specimens or kit reagents are handled.

STORAGE AND STABILITY
- Store the detector buffer in a refrigerator at 2° - 8°C. The Detector Buffer is stable up to 20 months if stored in a refrigerator.
- Moved from refrigerator, allow the Detector Buffer for 30 minutes to return to room temperature before testing.
- Store i-CHROMA™ HCG Device at 4°-30°C in its sealed pouch. The i-CHROMA™ hCG Device is stable for 20 months (while in the sealed pouch) if stored at 4°-30°C.
- If stored in a refrigerator, allow a minimum of 30 minutes for the test device and detection buffer to reach room temperature while it is in the sealed pouch.
- Do not remove the device from the pouch until ready to use. The Test Device should be used immediately once opened.
- The storage and shipping of Test Kit should be complied as indicated in manual. However, it is remotely possible that only part of Test Kit is affected by stability problems.

SAMPLE COLLECTION AND PREPARATION
The test can be performed with either serum or plasma.

- For serum sample, collect the blood in a tube without anticoagulant and allow to clot. Remove the serum from the clot as soon as possible to avoid hemolysis. For plasma sample, collect the blood in a tube treated with EDTA. Anticoagulants other than EDTA for plasma specimens have not been evaluated. If testing cannot be conducted within an hour after preparation of

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specimen, the serum/plasma should be stored at -20°C until tested. In case of use, apply it immediately after specimen was taken.

• The specimen must be at room temperature and be homogeneous before testing. Frozen specimens must be completely thawed, thoroughly mixed, and brought to room temperature prior to testing. If specimens are to be shipped, they should be packed in compliance with regulations.

• It is recommended to avoid using severely hemolyzed specimens whenever possible. If a specimen appears to be severely hemolyzed, another specimen should be obtained and tested.

MATERIALS PROVIDED
BodiTech Med Incorporated i-CHROMA™ hCG
Catalog TM REF Catalog No. 13009

Kit contains:
Test Devices 25T/box
ID Chip 1 ea/box
Detection Buffer 25 tubes/pouch

MATERIALS REQUIRED BUT NOT PROVIDED
i-CHROMA™ Reader REF Catalog No. FR-203
Thermal Printer
Transfer pipette (75µL size)

PROCEDURE
• Image of the test kit

1. Set a Test Device on a dust-free clean place.
2. Check/insert ID Chip onto the instrument. Make sure that the Test Device lot # matches with ID Chip #.
3. Take out one tube of Detection Buffer from refrigerator and leave it at room temperature.
4. Draw 75 µL of serum/plasma or control with a transfer pipette and add it to the tube containing Detector Buffer.
5. Mix well the specimen with Detector Buffer by tapping or inverting the tube.
6. Take 75 µL of sample mixture and load it onto the well of disposable Test Device.
7. Leave the Test Device at room temperature for 15min before inserting the device into the holder.
8. Insert Test Device onto the holder of i-CHROMA™ Reader. And press “SELECT” button. Make sure direction of Test Device and push the device back all the way.
9. Read the results on the display screen of i-CHROMA™ Reader.

➢ Refer to i-CHROMA™ Reader Operation Manual for the complete instructions on use of the Test.

RESULT
The i-CHROMA™ Reader calculates hCG test results automatically and displays concentration of hCG in blood sample on the LCD as form of mIU/mL.

Quality Control
A quality control test using commercially available controls should be performed as part of good testing practice, to confirm the expected QC results, to confirm the validity of the assay, and to assure the accuracy of patient results. If you want to perform QC of Test Kit, we recommend using Lyphochek® Immunoassay Plus Control.

• A quality control test should be performed at regular intervals, and before using a new kit with patient specimens, controls should be tested to confirm the test procedure, and to verify the tests produce the expected QC results. QC specimens should also be run whenever there is any question concerning the validity of results obtained. Upon confirmation of the expected results, the test device is ready to use with patient specimens. Control standards are not provided with this test kit. For information about obtaining the controls, contact BodiTech Med Incorporated’s Technical Services for assistance.

Procedure Control
• Each i-CHROMA™ hCG Device contains internal control that satisfies routine quality control requirements. This internal control is performed each time a patient sample is tested. This control indicates that the test device was inserted and read properly by i-CHROMA™ Reader. An invalid result from the internal control causes an error message on i-CHROMA™ Reader indicating that the test should be repeated.

LIMITATIONS OF THE PROCEDURE
• The results of i-CHROMA™ hCG should be evaluated with all clinical and laboratory data available. If hCG Test results do not agree with the clinical evaluation, additional tests should be performed.

• The false positive results include cross-reactions with some components of serum from individual to antibodies; and non-specific adhesion of some components in human blood that have similar epitopes to capture and detector antibodies. In the case of false negative results, the most common factors are: non-responsiveness of antigen to the antibodies by that certain unknown components are masking its epitope, such that antigen cannot be seen by the antibodies; instability of hCG antigen, resulting in degradation with time and, or temperature, such that they become no longer recognizable by antibodies; and degraded other test components. The effectiveness of the test is highly dependent on storage of kits and sample specimens at optimal conditions.

• Plasma using anticoagulants (e.g. heparin or citrate) other than EDTA has not been evaluated in i-CHROMA™ hCG Test and thus should not be used.

• Other factors may interfere with i-CHROMA™ hCG Test and may cause erroneous results. These include technical or procedural errors, as well as additional substances in blood specimens.

Performance Characteristics
1. Analytical Sensitivity
Analytical sensitivity means the lowest concentration of hCG that the test system can detect with CV<10%. Analytical sensitivity of i-CHROMA™ hCG Test was determined by testing 10 times each using 3 lots of reagents. Analytical sensitivity of i-CHROMA™ hCG was 5 mIU/ml.
2. **Specificity**

Other bio-molecules, such as LH(300mIU/ml), FSH (1,000mIU/ml), and TSH(1,000 μIU/ml) were added to test specimen with much higher level than their physiological level in normal blood. There was no significant interference with the hCG measurement, nor was their any significant assay cross-reactivity with those bio-molecules tested.

3. **Interfering Substances**

The following potentially interfering substances were added to hCG negative and positive specimens.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Concentration (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetaminophen</td>
<td>20</td>
</tr>
<tr>
<td>Acetylsalicylic Acid</td>
<td>20</td>
</tr>
<tr>
<td>Ascorbic Acid</td>
<td>20</td>
</tr>
<tr>
<td>Atropine</td>
<td>20</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>20</td>
</tr>
<tr>
<td>Caffeine</td>
<td>20</td>
</tr>
<tr>
<td>Gentisic Acid</td>
<td>20</td>
</tr>
<tr>
<td>Glucose</td>
<td>2</td>
</tr>
<tr>
<td>Hemoglobin</td>
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</tr>
</tbody>
</table>

None of the substance at the concentration tested interfered in the assay.

4. **Precision**

<table>
<thead>
<tr>
<th>Sample (mIU/ml)</th>
<th>Intra-assay Mean (mIU/ml)</th>
<th>CV (%)</th>
<th>Inter-assay Mean (mIU/ml)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>14.6</td>
<td>14.2</td>
<td>7.2</td>
<td>14.3</td>
<td>7.7</td>
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<tr>
<td>39.5</td>
<td>39.9</td>
<td>5.2</td>
<td>39.2</td>
<td>5.6</td>
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<td>1208.2</td>
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<td>6462.4</td>
<td>3.9</td>
<td>6487.3</td>
<td>4.5</td>
</tr>
</tbody>
</table>

5. **Comparability**

hCG concentrations of 30 clinical specimens were quantified independently with *i-CHROMA™ hCG* kit and Bayer Centaur automatic analyzer according to established standard test procedure. Test result was compared and their compatibility was investigated with linear regression and correlation of coefficient ($R$). Linear regression and correlation of coefficient were $Y=1.03X-2.9285$ and $R=0.998$, respectively.

![Graph showing the linear relationship between i-CHROMA hCG conc. and Centaur hCG conc.](image)

**REFERENCES**