DIRECTIONS FOR USE

Allow reagents and specimens to reach room temperature (20-30°C) prior to testing. The procedure must be followed. Assay must proceed to completion within time limits. Arrange the control so that well A1 is the Blank well. From well A1, arrange the Calibrator in a horizontal or vertical ascending order, ensuring specific wells arranged in a vertical configuration. Configuration may depend upon software.

Materials Required But Not Provided
- Calibrated micropipettes with disposable tips
- Vortex mixer for specimen mixing (optional)
- Graduated cylinders for wash buffer dilution
- Water bath or incubator capable of maintaining 37°C
- Disposable reagent reservoirs
- Disposable microcentrifuge tubes
- Disposable gloves
- Automated processor (optional)

PROCEDURE

1. Prepare Working Wash Buffer by diluting the Concentrated Wash Buffer 1:25. Prepare Working Working Wash Buffer by diluting the Concentrated Wash Buffer 1:25 and store in the original resealable pouch at 2-8°C.

2. Prepare Working Wash Buffer by diluting the Concentrated Wash Buffer 1:25 and store in the original resealable pouch at 2-8°C.

3. Add 20 μL of specimen to assigned wells start at D2. Place specimen accordingly after the working solution is prepared.

4. Add 20 μL specimen to each well except for the Blank well (Red Reagent).

5. Add 100 μL of Conjugate to each well except for the Blank well.

6. Prepare Working Wash Buffer by diluting the Concentrated Wash Buffer 1:25.

7. Reserve and store unused washes at 2-8°C.

Note: If crystals are present in the Concentrated Wash Buffer, warm it up to 37°C until all crystals dissolve. Remove unused strips from the microplate strip, and discard well plate prior to use. Do not re-use washable strips in a reusable washable pouch at 2-8°C.

Prepare Working Wash Buffer by diluting the Concentrated Wash Buffer 1:25.
AUTOMATED PROCESSING

Automatic EIA microplate processors may be used to perform the assay after validating the results to ensure they are equivalent to those obtained using the manual method for the same specimen. Incubation times may vary depending on the processors used but do not program less incubation times than the procedure listed above. When automatic EIA microplate processors are used, periodic validation is recommended to ensure proper results.

VALIDATION REQUIREMENTS AND QUALITY CONTROL

1. Calculate the Mean Absorbance of Calibrators 1-5 by referring to the table below. Example of Calibrator 2 Calculation

<table>
<thead>
<tr>
<th>Item</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calibrator 2: Well D1</td>
<td>0.404</td>
</tr>
<tr>
<td>Calibrator 2: Well E1</td>
<td>0.398</td>
</tr>
<tr>
<td>Total Absorbance of Calibrator 2</td>
<td>0.802</td>
</tr>
<tr>
<td>Mean Absorbance of Calibrator 2</td>
<td>0.401</td>
</tr>
<tr>
<td>Blank Absorbance: Well A1</td>
<td>0.028</td>
</tr>
<tr>
<td>Mean Absorbance of Calibrator 2 - Blank Absorbance</td>
<td>0.373</td>
</tr>
</tbody>
</table>

2. Check the validation requirements below to determine if the test results are valid.

- Mean Absorbance of each Calibrator should be < 0.500 if read at 450/630-700 nm
- Total Absorbance of each Calibrator should be < 0.500

NOTE: The test results are considered invalid if the above validation requirements are not met. Repeat the test or contact your local distributor.

INTERPRETATION OF RESULTS

1. Subtract the Blank Absorbance from the Mean Absorbance of each Calibrator, and then plot them on the Y-axis against their concentration in mIU/mL on the X-axis on a linear graph paper and draw the calibration curve. Draw the best fitted line through data points to obtain a standard curve. Refer to the calibration curve at right.

- **NOTE:** Do not use the calibration curve above to make any calculation. A calibration curve must be performed for each run.
- 2. Obtain quantitative specimen results from their absorbance after subtraction of Blank Absorbance by using the calibration curve.

1. It is recommended that for visual qualitative detection to interpret the results directly without adding the stop solution. Blue color is easier for interpretation than the yellow color which will be produced after the addition of Stop Solution. In this case, make sure to interpret the results immediately. Delay in the interpretation may cause false positive.

2. Compare the color intensity of the specimen wells to the color intensity of the wells for Calibrator 2 (25 mIU/mL).

3. If color intensity of the specimen wells is lower than the color intensity of the wells for Calibrator 2, meaning the hCG concentration of the specimen is lower than 25 mIU/mL, then the specimen should be considered as Negative.

4. If color intensity of the specimen wells is higher than the color intensity of the wells for Calibrator 2, meaning the hCG concentration of the specimen is higher than 25 mIU/mL, then the specimen should be considered as Positive.

LIMITATIONS

1. The Rapid hCG EIA Test Kit is used for the detection of hCG in human serum or urine.

2. As with all diagnostic immunoassays, there is the possibility that non-repeatable reactive results may occur due to inadequate washing. The results may be affected due to procedural or instrument error.

3. Enzyme reaction may be due to fibrin particles and microbial contamination.

PERFORMANCE CHARACTERISTICS

The analytical sensitivity of the Rapid hCG EIA Test Kit is 0.68 mIU/mL.

Intra-Assay: Within-run precision has been determined by using 10 replicates of three specimens with hCG concentration at 50 mIU/mL, 100 mIU/mL, and 250 mIU/mL respectively.

Inter-Assay: Between-run precision has been determined by 3 independent assays on the same three specimens with hCG concentration at 50 mIU/mL, 100 mIU/mL, and 250 mIU/mL respectively. The Rapid hCG EIA Test Kit has been tested using the specimens over a 5-day period.

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Intra-Assay Mean Absorbance</th>
<th>Standard Deviation</th>
<th>Coefficient of Variation (%)</th>
<th>Inter-Assay Mean Absorbance</th>
<th>Standard Deviation</th>
<th>Coefficient of Variation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.389</td>
<td>0.039</td>
<td>8.64</td>
<td>0.987</td>
<td>0.123</td>
<td>12.46</td>
</tr>
<tr>
<td>2</td>
<td>0.856</td>
<td>0.074</td>
<td>8.64</td>
<td>0.987</td>
<td>0.123</td>
<td>12.46</td>
</tr>
<tr>
<td>3</td>
<td>1.234</td>
<td>0.187</td>
<td>15.02</td>
<td>1.756</td>
<td>0.176</td>
<td>10.12</td>
</tr>
</tbody>
</table>

The Rapid hCG EIA Test Kit has been compared to a leading commercial Rapid hCG EIA test using clinical specimens. A total of 66 serum and 70 urine specimens ranging from 0-250mIU/mL were run and analyzed using least square regression analysis. The results show that the Rapid hCG EIA Test Kit has good correlation compared to the reference method.

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Items</th>
<th>No.</th>
<th>Range (mIU/mL)</th>
<th>Slope</th>
<th>Correlation Coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Urine</td>
<td>70</td>
<td>0-250</td>
<td>0.53</td>
<td>0.94</td>
</tr>
<tr>
<td>2</td>
<td>HCG</td>
<td>100</td>
<td>0-250</td>
<td>0.53</td>
<td>0.94</td>
</tr>
</tbody>
</table>

No dose hook effect is observed up to 250000 mIU/mL of hCG.

HCG INTERFERENCE AND CROSS-REACTIVITY

The specificity of the Rapid hCG EIA Test Kit was determined by testing sera containing the compounds listed below. These compounds showed less than 20% interference in the Rapid hCG EIA Test Kit at the levels indicated.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Concentration</th>
<th>Substance Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>albumin</td>
<td>60 µg/mL</td>
<td>IgG</td>
</tr>
<tr>
<td>albumin</td>
<td>100 µg/mL</td>
<td>hCG</td>
</tr>
</tbody>
</table>

The following substances and concentrations have also been tested using Rapid hCG EIA Test Kit and no interference was observed.