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## Free Beta-Human Chorionic Gonadotropin (Free $\beta$ -hCG) ELISA

Catalog No.: HC365F (96 Tests)

### INTENDED USE

The Calbiotech, Inc. free  $\beta$ -hCG ELISA kit is intended for the quantitative determination of free beta subunit of hCG in serum or plasma. **For Research Use Only. For professional use only. Not for use in diagnostic procedures.**

### SUMMARY AND EXPLANATION

Detects the free beta subunit of human chorionic gonadotropin, a glycoprotein hormone produced by trophoblastic cells. Research uses include placental biology, glycoprotein hormone structure–function studies, and molecular regulation of hCG expression in normal and transformed cell lines.

### PRINCIPLE OF THE TEST

The Calbiotech free  $\beta$ -hCG kit is a solid phase sandwich assay method based on streptavidin-biotin principle. Standards, samples, and the biotinylated anti-free  $\beta$ -hCG antibody reagent are added into wells coated with Streptavidin. Free  $\beta$ -hCG in the samples binds to the biotinylated antibody. Simultaneously, the biotinylated antibody binds to the Streptavidin coated plate. Unbound protein and excess biotin conjugated antibody are washed off by wash buffer. Upon the addition of Peroxidase (HRP) conjugated anti-free  $\beta$ -hCG antibody reagent, a sandwich complex is formed, where the free  $\beta$ -hCG being in between the two highly specific antibodies, labeled with biotin and HRP. Unbound protein and excess enzyme conjugated antibody reagent is washed off by wash buffer. Upon the addition of the substrate, the intensity of color developed is directly proportional to the concentration of free  $\beta$ -hCG in the samples. A standard curve is prepared relating color intensity to the concentration of free  $\beta$ -hCG.

MATERIALS PROVIDED		96 TESTS
1.	Microwells coated with Streptavidin	12x8x1
2.	Free $\beta$ -hCG Standards: 6 vials (ready to use)	0.5 mL
3.	Free $\beta$ -hCG Controls: 2 vials (ready to use)	0.5 mL
4.	Anti-free $\beta$ -hCG Biotin Reagent: 1 bottle (ready to use)	12 mL
5.	Anti-free $\beta$ -hCG HRP Enzyme Conjugate: 1 bottle (ready to use)	12 mL
6.	TMB Substrate: 1 bottle (ready to use)	12 mL
7.	Stop Solution: bottle (ready to use)	12 mL
8.	Wash concentrate 20X: 1 bottle	25 mL

### MATERIALS NOT PROVIDED

1. Distilled or deionized water
2. Precision pipettes
3. Disposable pipette tips
4. ELISA reader capable of reading absorbance at 450 nm
5. Absorbance paper or paper towel
6. Graph paper

### STORAGE AND STABILITY

1. Store the kit at 2-8°C.
2. Keep microwells sealed in a dry bag with desiccants.
3. The reagents are stable until expiration of the kit.
4. Do not expose test reagents to heat, sun or strong light.



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**WARNINGS AND PRECAUTIONS**

Potential biohazardous materials:

1. The standards contain human source components which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, as there is no test method that can offer complete assurance that HIV, Hepatitis B virus or other infectious agents are absent, these reagents should be handled at the Biosafety Level 2, as recommended in the Centers for Disease Control/National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories." 1984.
2. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
3. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
4. It is recommended that standards, control and serum samples be run in duplicate
5. Optimal results will be obtained by strict adherence to this protocol. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from this may yield invalid data.

**SPECIMEN COLLECTION AND HANDLING**

This assay is designed for use with human serum or plasma samples obtained in compliance with applicable laws, regulations, and institutional policies. Handle and store samples using procedures appropriate for research use. Samples may be stored refrigerated (2–8 °C) for up to seven days, or frozen (–20 °C or below) for up to six months. Avoid repetitive freeze–thaw cycles.

**REAGENT PREPARATION**

1. Wash Buffer: Prepare 1X Wash Buffer by adding the contents of the bottle (25ml, 20X) to 475ml of distilled water. Store at room temperature (20-25°C).

**ASSAY PROCEDURE**

1. Place the desired number of coated strips into the holder.
2. Dispense 25µl free β-hCG standards, controls, and samples into appropriate wells.
3. Add 100µl of Biotin Reagent into all the wells. Shake the microplate gently for 20-30 seconds to mix.
4. Incubate for 30 minutes, at room temperature (20-25°C).
5. Briskly shake out the contents of the wells. Rinse the wells 3 times with 1X wash buffer. Strike the wells sharply on absorbent paper to remove residual water droplets.
6. Add 100µl of HRP Enzyme Conjugate to all the wells.
7. Incubate for 15 minutes, at room temperature (20-25°C).
8. Briskly shake out the contents of the wells. Rinse the wells 3 times with 1X wash buffer. Strike the wells sharply on absorbent paper to remove residual water droplets.
9. Add 100µl of TMB substrate to all the wells.
10. Cover the microplate and incubate for 15 minutes, at room temperature.
11. Add 50µl of stop solution to each well and gently mix until a uniform color, in each well, is obtained.
12. Read the absorbance in each well at 450nm within 15 minutes after adding the stop solution.

**CALCULATION OF RESULTS**

A standard curve is constructed as follows:

1. Calculate the average absorbance values for each set of standards and patient samples.
2. To construct the standard curve, plot the mean absorbance of each free β-hCG standards (vertical axis) against its concentration in ng/ml (horizontal axis).
3. Draw the best-fit curve through the plotted points.
4. Read the absorbance for each unknown sample from the curve to determine the corresponding concentration of free β-hCG.

**Example of a Typical Standard Curve**

	OD450nm	Conc. (ng/mL)
<b>Std 1</b>	0.008	0
<b>Std 2</b>	0.087	5
<b>Std 3</b>	0.170	10
<b>Std 4</b>	0.458	25
<b>Std 5</b>	1.482	100
<b>Std 6</b>	2.603	250