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Free Beta-Human Chorionic Gonadotropin (Free β-hCG) ELISA Kit

Name	Free Beta-Human Chorionic Gonadotropin (Free β-hCG) ELISA Kit
INTENDED USE	The free β-hCG ELISA kit is intended for the quantitative determination of free beta subunit of hCG in serum or plasma.
PRINCIPLE OF THE TEST	The free β-hCG kit is a solid phase sandwich assay method based on streptavidin-biotin principle. Standards, samples, and the biotinylated anti-free β-hCG antibody reagent are added into wells coated with Streptavidin. Free β-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 β-hCG antibody reagent, a sandwich complex is formed, where the free β-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 β-hCG in the samples. A standard curve is prepared relating color intensity to the concentration of free β-hCG.

MATERIALS PROVIDED	96 Tests
Microwells coated with Streptavidin	12x8x1
Free β-hCG Standards: 6 vials (ready to use)	0.5 ml
Anti-free β-hCG Biotin Reagent: 1 bottle (ready to use)	12 ml
Anti-free β-hCG HRP	12 ml

Enzyme Conjugate: 1 bottle (ready to use)	
TMB Substrate: 1 bottle (ready to use)	12 ml
Stop Solution: bottle (ready to use)	12 ml
Wash concentrate 20X: 1 bottle	25 ml

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MATERIALS NOT PROVIDED

Distilled or deionized water

Precision pipettes

Disposable pipette tips

ELISA reader capable of reading absorbance at 450 nm

Absorbance paper or paper towel

Graph paper

STORAGE AND STABILITY

Store the kit at 2-8oC.

Keep microwells sealed in a dry bag with desiccants.

The reagents are stable until expiration of the kit.

Do not expose test reagents to heat, sun or strong light.

WARNINGS AND PRECAUTIONS

Potential biohazardous materials:

The calibrator and controls 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, 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.

Optimal results will be obtained by strict adherence to the test protocol. Precise pipetting as well as following the exact time and temperature requirements is essential.

Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.

The components in this kit are intended for use as an

	integral unit. The components of different lots should not be mixed.
SPECIMEN COLLECTION AND HANDLING	Collect blood specimens and separate the serum immediately. Specimens may be stored refrigerated at (2-8C) for 5 days. If storage time exceeds 5 days, store frozen at (-20 C) for up to one month. Avoid multiple freeze-thaw cycles. Prior to assay, frozen sera should be completely thawed and mixed well. Do not use grossly lipemic specimens.
REAGENT PREPARATION	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	Place the desired number of coated strips into the holder. Dispense 25µl free β-hCG standards, controls, and samples into appropriate wells. Add 100µl of Biotin Reagent into all the wells. Shake the microplate gently for 20-30 seconds to mix. Incubate for 30 minutes, at room temperature (20-25°C). 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. Add 100µl of HRP Enzyme Conjugate to all the wells. Incubate for 15 minutes, at room temperature (20-25°C). 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. Add 100µl of TMB substrate to all the wells. Cover the microplate and incubate for 15 minutes, at room temperature. Add 50µl of stop solution to each well and gently mix until a uniform color, in each well, is obtained. 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:

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Calculate the average absorbance values for each set of standards and patient samples.

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).

Draw the best-fit curve through the plotted points.

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)
Std 1	0.008	0
Std 2	0.087	5
Std 3	0.170	10
Std 4	0.458	25
Std 5	1.482	100
Std 6	2.603	250

EXPECTED VALUES

We recommend each laboratory to establish its own normal ranges, for the population it serves. Until then, literature values may be used as guidelines.

REPORTABLE RANGE: Analytical Range = 5 - 250 ng/ml

Samples that fall within the calibration curve should be reported as such. Samples that fall outside the calibration curve should be reported 250 ng/ml.

Sensitivity

The sensitivity was determined by calculating the mean plus 2SD of the standard zero point tested 24 times in the same run.

Serum	No. of Replicates	Mean (ng/mL)	Standard Deviation	Mean + 2SD (Sensitivity)
Zero Standard	24	0.04	0.05	0.15

Precision

Intra-Assay

Serum	No. of	Mean	Standard	Coefficien

	Replicates	(ng/mL)	Deviation	t of Variation (%)
1	24	5.7	0.24	4.3
2	24	45.9	1.52	3.3
3	24	126.3	6.11	4.8

Inter-assay

Serum	No. of Replicates	Mean ng/ml	Standard Deviation	Coefficient of Variation (%)
1	24	5.5	0.25	4.6
2	24	43.7	1.32	3.0
3	24	120.4	4.47	3.7