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For Research Use Only. Not for use in Diagnostic Procedures.

Manufactured for:

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Human Chorionic Gonadotropin (hCG) ELISA

Catalog No. IB19115 (96 Tests)

INTENDED USE

The hCG ELISA Kit is intended for the measurement of hCG in human serum or plasma.
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SUMMARY AND EXPLANATION

Human Chorionic Gonadotropin (hCG) is a 40 kD glycoprotein hormone secreted by the placenta. hCG has two subunits, alpha and beta. The alpha subunit is similar to the alpha subunit found in LH, FSH and TSH glycoprotein hormones. However, the beta subunit is specific and differs from hormone to hormone.
The serum hCG rises in early pregnancy to concentrations of 50,000-150,000 mIU/ml between the 8th and 12th weeks of gestation and decline to 20,000 mIU/ml by the 18th week where they remain for the duration of the pregnancy. The increased level of hCG in non-pregnant women or men suggest neoplasia. Thus hCG measurement is useful for the recognition and monitoring of chorionic tumors and as a tumor marker for other malignancies that produce hCG ectopically. These include testicular, pancreatic, and bronchogenic pulmonary cancers.

PRINCIPLE OF THE TEST

The hCG ELISA is an adapted solid phase sandwich ELISA method. The samples, biotin labeled anti-hCG and anti-hCG-HRP conjugate are added to the wells coated with Streptavidin. hCG in the serum binds to the anti-hCG antibodies and forms a sandwich on the streptavidin coated plate. Unbound protein and HRP conjugate are washed off by wash buffer. Upon the addition of the substrate, the intensity of color is proportional to the concentration of hCG in the samples. A standard curve is prepared relating color intensity to the concentration of hCG in the sample.

	MATERIALS PROVIDED	96 Tests
1.	Microwells coated with Streptavidin	12x8x1
2.	hCG Standards: 6 vials (ready to use)	0.5ml
3.	hCG Controls: 2 vials (ready to use)	0.5ml
4.	hCG Conjugate Reagent: 1 bottle (ready to use)	12 ml
5.	TMB Substrate: 1 bottle (ready to use)	12ml
6.	Stop Solution: 1 bottle (ready to use)	12ml
7.	20X Wash concentrate: 1 bottle	25ml

MATERIALS NOT PROVIDED

1. Distilled or deionized water
2. Precision pipettes
3. Disposable pipette tips
4. ELISA reader capable of reading absorbance at 450nm
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.

WARNINGS AND PRECAUTIONS

1. 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, 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. This test kit is designed for research use only.
3. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
4. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
5. It is recommended that serum samples be run in duplicate.
6. 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 HANDLING

1. Collect blood specimens and separate the serum immediately.
2. Specimens may be stored refrigerated at (2-8°C) for 5 days. If storage time exceeds 5 days, store frozen at (-20° C) for up to one month.
3. Avoid multiple freeze-thaw cycles.
4. Prior to assay, frozen sera should be completely thawed and mixed well.
5. Do not use grossly lipemic specimens.

REAGENTS PREPARATION

Prepare 1X Wash buffer by adding the contents of the bottle (25 ml, 20X) to 475 ml of distilled or deionized water. Store at room temperature (18-26° C).

ASSAY PROCEDURE

Prior to assay, bring all reagents to room temperature (18°-26°C).
Gently mix all reagents before use.

1. Place the desired number of coated strips into the holder. Replace any unused microwell strips back into the foil pouch, seal, and store at 2-8°C.
2. Pipet 25 µl of hCG standards, controls, and samples in to appropriate wells.
3. Add 100 µl of Conjugate Reagent to all wells.
4. Incubate for 60 minutes at room temperature (18-26° C).
5. Remove liquid from all wells. Wash wells three times with 300 µl of 1X wash buffer. Blot on absorbent paper towels.
6. Add 100 µl of TMB substrate to all wells.
7. Incubate for 15 minutes at room temperature.
8. Add 50 µl of stop solution to all wells. Shake the plate gently to mix the solution.
9. Read absorbance on ELISA Reader at 450 nm within 15 minutes after adding the stopping solution.

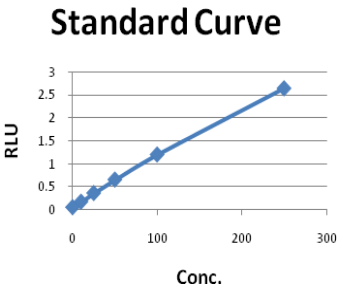
CALCULATION OF RESULTS

The standard curve is constructed as follows:

1. Standard values may vary slightly with each lot. Be sure to use the correct value (printed on the vial label and included with the Certificate of Analysis. The standard below is only an example**.**
2. To construct the standard curve, plot the absorbance for the hCG standards (vertical axis) versus the hCG standard concentrations in mIU/ml (horizontal axis) on a linear graph paper. Draw the best curve through the points.
3. Read the absorbance for controls and each unknown sample from the curve. Record the value for each control or unknown sample.
4. Values above 250 mIU should be retested after diluting with “0” standard.

Example of a Standard Curve

Standard	OD (450 nm)
Standard 1 (0 mIU/ml)	0.048
Standard 2 (10 mIU/ml)	0.169
Standard 3 (25 mIU/ml)	0.357
Standard 4 (50 mIU/ml)	0.650
Standard 5 (100 mIU/ml)	1.198
Standard 6 (250 mIU/ml)	2.642



LIMITATIONS OF THE TEST

1. Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities.