

**CALDON BIOTECH INC.**  
**Human Chorionic**  
**Gonadotropin (hCG)**  
**ELISA**

Catalog No. HC047F  
 (96 tests)

**INTENDED USE**

The CALDON BIOTECH INC. (CBI) hCG ELISA kit is used for the quantitative measurement of hCG in human serum or plasma.

**CLINICAL UTILITY**

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 8<sup>th</sup> and 12<sup>th</sup> weeks of gestation and decline to 20,000 mIU/mL by the 18<sup>th</sup> 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. The sensitivity of this ELISA test is 0.5mIU/mL.

**PRINCIPLE OF THE TEST**

The CBI hCG is a direct solid phase sandwich ELISA method. The samples and diluted anti-hCG-HRP conjugate are added to the wells coated with Mab to beta subunit. hCG in the patient's serum binds to anti-hCG MAb on the well and the anti-hCG second antibody then binds to hCG. 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 the hCG.

**MATERIALS PROVIDED**

1. Microwell strips coated with hCG MAb (12x8x1 wells). Total of 96 wells.
2. hCG Standard: 6 vials (0.7 mL). Ready to use.
3. hCG Enzyme Conjugate: 1 bottle (12 mL). Ready to use

4. TMB Substrate: 1 bottle (12 mL). Ready to use.
5. Stop Solution: 1 bottle (8 mL). Ready to use.
6. 10X Wash Concentrate: 50 mL.

**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.

**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 in vitro diagnostic 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**

10X Wash Buffer Concentrate: To prepare working wash buffer, add the contents of the bottle to 450 ml of distilled water. Store at room temperature.

**ASSAY PROCEDURE**

Prior to assay, allow reagents to stand at room temperature.

Gently mix all reagents before use.

1. Place the desired number of coated strips into the holder
2. Pipet 50  $\mu$ L of hCG standards, control and patient's sera.
3. Add 100  $\mu$ L of Enzyme Conjugate to all wells.
4. Cover the plate and incubate for 60 minutes at room temperature (18-26° C).
5. Remove liquid from all wells. Fill wells with working wash buffer. Wash three times. Blot on absorbent paper towels.
6. Add 100  $\mu$ L of TMB substrate to all wells.
7. Incubate for 10 minutes at room temperature.
8. Add 50  $\mu$ L of stop solution to all wells. Shake the plate gently to mix the solution.
9. Read absorbance on ELISA Reader at 450 nm within 20 minutes after adding the stopping solution.

**CALCULATION OF RESULTS**

The standard curve is constructed as follows:

1. Check hCG standard value on each standard vial. This value might vary from lot to lot. Make sure you check the value on every kit. See example of the standard attached.
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. Value above the highest point of the standard can be retested after diluting with "0" standard.

**EXPECTED VALUES**

It is recommended that each laboratory establish its own normal ranges based on a representative sampling of the local population. The following

values for hCG may be used as initial guideline ranges only:  
hCG Normal Range =Less Than 5 mIU/mL.

**LIMITATIONS OF THE TEST**

1. The test results obtained using this kit serve only as an aid to diagnosis and should be interpreted in relation to the patient's history, physical findings and other diagnostic procedures.
2. Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities.

**PERFORMANCE CHARACTERISTICS**

**1. Correlation with a Reference ELISA kit:**

A total of 125 sera were tested by this ELISA and a reference ELISA kit. Results were as follows:

Correlation	Slope	Intercept
0.95	0.89	1.95

**2. Precision Intra-Assay**

Serum	No. of Replicates	Mean mIU/mL	Standard Deviation	Coefficient of Variation %
Normal	16	141.5	5.40	3.82
	16	31.1	1.41	4.53
	16	8.4	0.89	10.59

**Inter-assay**

Serum	No. of Replicates	Mean mIU/mL	Standard Deviation	Coefficient of Variation %
Normal	10	153	17.3	11.30
	10	26.8	3.29	12.27
	10	8.6	1.36	15.8

**3. Sensitivity**

The sensitivity was determined by calculating the mean plus 2SD of the standard zero point tested 20 times in the same run.

Serum	No. of Replicates	Mean mIU/mL	Standard Deviation	Mean + 2SD (Sensitivity)
Zero Standard	20	0.12	0.205	0.53 mIU/mL

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1991;39(2):117-22.

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#### 4. Recovery

Known quantities of hCG were added to a serum that contained a low concentration of hCG.

Expected Value (mIU/mL)	Recovered (mIU/mL)	Percentage of Recovery
61.2	62.0	101
75.8	71.8	94.7
242.0	247.0	102

#### 5. Linearity

Three different patient samples were diluted with the "0" calibrator to 1:2, 1:4 and 1:8. hCG values were assayed and results were corrected with the dilution factor. The results of these dilution tests are as follows:

Serum	Original Value (mIU/mL)	Percentage of Recovery		
		1:2	1:4	1:8
1	56	94	92	101
2	106	108	120	99
3	144	114	114	107

#### REFERENCES:

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4. Mantzavinos T; Phocas I; Chrelas H; Sarandakou A; Zourlas PA. Serum levels of steroid and placental protein hormones in ectopic pregnancy. Eur J