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Product Information

Human Chorionic Gonadotropin (hCG) ELISA

Catalog Number **SE120063**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description

Human Chorionic Gonadotropin (hCG) is a 40 kDa 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.

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 neoplsia. 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 hCG ELISA Kit is intended for the quantitative measurement of hCG in human serum or plasma. The hCG is a direct solid phase sandwich ELISA method. The samples and diluted anti-hCG-HRP conjugate are added to the wells coated with a monoclonal antibody (MAb) to beta subunit. hCG in the 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. The sensitivity of this ELISA test is 0.5 mIU/mL.

Components

Materials Provided	96 Tests
Microwells coated with hCG MAb	12 x 8 x 1
hCG Standards: 6 vials (ready to use)	0.5 mL
hCG Enzyme Conjugate: 1 bottle (ready to use)	12 mL
TMB Substrate: 1 bottle (ready to use)	12 mL
Stop Solution: 1 bottle (ready to use)	12 mL
20x Wash concentrate: 1 bottle	25 mL

Reagents and Equipment Required but Not Provided.

- Distilled or deionized water
- Precision pipettes
- Disposable pipette tips
- ELISA reader capable of reading absorbance at 450 nm
- Absorbent paper or paper towel
- Graph paper

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Sample Preparation

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.

20x Wash Buffer Concentrate
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).

Storage/Stability
Store the kit at 2–8 °C.

Procedure
Notes: The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.

It is recommended that serum samples be run in duplicate.

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

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.

Bring all specimens and kit reagents to room temperature (18-26 °C) and gently mix.

1. Place the desired number of coated strips into the holder
2. Pipette 50 µL of hCG standards, control, and sera.
3. Add 100 µL 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. 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 10 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.

Results

Calculations

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 the value is checked on every kit.
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.

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

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 are 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 <5 mIU/mL

Note: 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.

References

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2. Choi, M.J. et al., Simple enzyme immunoassay for the simultaneous measurement of whole choriogonadotropin molecules and free beta-subunits in sera of women with abnormal pregnancies or tumors of the reproductive system. Clin. Chem., 1991;37(5):673-7.
3. Trundle, D.S. et al., Automated determination of human choriogonadotropin by use of microparticle capture analysis. Clin. Chem., 1990;36(3):554-6.
4. Mantzavinos, T. et al., Serum levels of steroid and placental protein hormones in ectopic pregnancy. Eur. J. Obstet. Gynecol. Reprod. Biol., 1991; 39(2): 117-22.

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