Human beta hCG ELISA Kit

Enzyme Immunoassay for the quantification of human beta hCG in serum samples

Catalog number: ARG80835

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INTRODUCTION

Chorionic Gonadotropin (hCG) is a glycoprotein hormone which is normally produced by the placenta during pregnancy. After conception, the hCG concentration increases rapidly to reach a peak near the end of the first trimester. High concentrations are observed throughout pregnancy. After delivery, hCG levels fall rapidly and become undetectable after a few days.

Structurally intact hCG molecules are composed of an alpha and a beta subunit. The alpha subunit is nearly identical to the alpha subunits of other glycoprotein hormones, such as Thyroid Stimulating Hormone (TSH), Luteinizing Hormone (LH), and Follicle Stimulating Hormone (FSH): The differences in the beta subunit of the respective hormones account for their biological specificity and immunochemical distinctiveness.

Monoclonal antibodies recognizing unique sites on the beta chain of the ß-hCG/hCG molecule are essential for differentiation between hCG and LH, FSH and TSH. Specific assays for beta-hCG permit the early detection of pregnancy.

In addition to the elevated hCG levels during pregnancy, high concentrations of ß-hCG/hCG may be associated with neoplasms of trophoblastic and nontrophoblastic origin such as hydatiform mole, chorionepithelioma, embryonal cell carcinoma,seminoma and many others.

PRINCIPLE OF THE ASSAY

This assay employs the sandwich enzyme immunoassay technique. A highly specific beta hCG antibody has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any beta hCG present is

bound by the immobilized antibody. After washing away any unbound substances, a HRP-antibody specific for beta hCG is added to each well and incubate. After washing away any unbound antibody-enzyme reagent, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of beta hCG bound in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450 nm ±2 nm. The concentration of beta hCG in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody-coated microplate	1 plate	4°C.
Standards 0-5 (0, 5, 25, 50, 100, 200 mIU/ml)	6 vials	4°C, lyophilized
HRP-antibody conjugate	11 ml (ready to use)	4°C
Sample Diluent	10 ml (ready to use)	4°C
TMB substrate	14 ml	4°C (Protect from light)
STOP solution	14 ml	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water

- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.
- Briefly spin down the HRP-antibody conjugate before use.
- If crystals are observed in the sample diluent buffer, warm to RT (not more than 50°C) until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Do not use haemolytic, icteric or lipaemic specimens.

Samples containing sodium azide should not be used in the assay

REAGENT PREPARATION

- **Standards:** Reconstitute the standards with 1 ml distilled water.
- **Samples:** Dilute 1:1000 with Sample Diluent buffer.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add 25 µl of each Standard, Control and samples into appropriate wells.
3. Add 100 µl of HRP-antibody conjugate to all wells.
4. Cover wells and incubate for 1 hour at RT.
5. Aspirate each well and wash, repeating the process 4 times for a total 5 washes. Wash by filling each well with distilled water (400 µl) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
6. Add 100 µl of TMB Reagent to each well. Incubate for 15 minutes at room temperature in dark.
7. Add 50 µl of Stop Solution to each well.
8. Read the OD with a microplate reader at 450 nm immediately.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls

and patient samples.

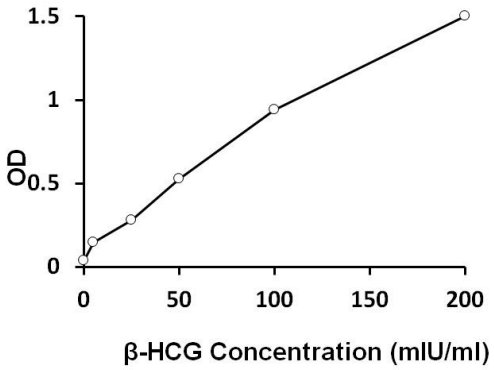
2. Using linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

Sensitivity

The analytical sensitivity was calculated from the mean plus two standard deviations of twenty (20) replicate analyses of the Standard 0 and was found to be < 1 mIU/ml.

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 5.43% and inter-assay precision was 7.93%.

Specificity

Protein	Concentration	Produced Color Intensity Equivalent to beta hCG in serum (mIU/mL)
hLH	300 mIU/ml	6
	200 mIU/ml	< 5
	80 mIU/ml	< 5
TSH	75 µIU/ml	8
	50 µIU/ml	< 5
	25 µIU/ml	< 5
FSH	200 mIU/ml	< 5
	50 mIU/ml	< 5

Recovery

89-100%