Product Manual

Revision date: 27/Feb/2017

Human beta HCG ELISA kit

Catalog No.: abx050244

Size: 96T

Range: 0 mIU/ml - 240 mIU/ml

Sensitivity: < 2 mIU/ml

Storage: Store at 2-8°C for 6 months.

Application: For quantitative detection of beta HCG in Human Serum and urine.

Introduction: Human chorionic gonadotropin (hCG) is a hormone produced by the placenta after implantation. Human chorionic gonadotropin is a glycoprotein composed of 237 amino acids with a molecular mass of 25.7 kDa. It is heterodimeric, with an α (alpha) subunit identical to that of luteinizing hormone (LH), follicle-stimulating hormone (FSH), thyroid-stimulating hormone (TSH), and β (beta) subunit that is unique to hCG. The α (alpha) subunit is 92 amino acids long. The β-subunit of hCG gonadotropin (beta-hCG) contains 145 amino acids, encoded by six highly homologous genes that are arranged in tandem and inverted pairs on chromosome 19q13.3. The two subunits create a small hydrophobic core surrounded by a high surface area-to-volume ratio: 2.8 times that of a sphere. The vast majority of the outer amino acids are hydrophilic. The hormone is produced by the placenta after implantation and some cancerous tumours. Therefore, elevated levels measured when the patient is not pregnant can lead to a cancer diagnosis and, if high enough, paraneoplastic syndromes. However, it is not known whether this production is a contributing cause or an effect of carcinogenesis. Regarding endogenous forms of hCG, there are various ways to categorize and measure them, including total hCG, C-terminal peptide total hCG, intact hCG, free β-subunit hCG, β-core fragment hCG, hyperglycosylated hCG, nicked hCG, alpha hCG, and pituitary hCG.

Principle of the Assay

This kit is based on sandwich enzyme-linked immuno-sorbent assay technology. Anti-beta HCG antibody is pre-coated onto 96-well plates. An HRP conjugated antibody is used as detection antibody. The standards, test samples and HRP conjugate reagent are added to the wells and incubated. Unbound conjugates are washed away with wash buffer. TMB substrate is used to visualize HRP enzymatic reaction. TMB is catalyzed by HRP to produce a blue color product that changes into yellow after adding acidic stop solution. The intensity of the color yellow is proportional to the beta HCG amount bound on the plate. The O.D. absorbance is measured spectrophotometrically at 450nm in a microplate reader, and then the concentration of beta HCG can be calculated.

Kit components

1. One 96-well plate
2. Human beta HCG Standard: 0.5 ml
3. Standard diluent buffer: 1.5 ml
4. Wash buffer (30×): 20 ml. Dilution: 1/30
5. Sample diluent buffer: 6 ml
6. HRP Conjugate Reagent: 6ml
7. Stop solution: 6 ml
8. TMB substrate A: 6ml
9. TMB substrate B: 6ml
10. Plate sealer: 2
11. Hermetic bag: 1

Material Required But Not Provided

1. 37°C incubator
2. Microplate reader (wavelength: 450nm)
3. Precision pipette and disposable pipette tips
4. Automated plate washer
5. ELISA shaker
6. 1.5ml tubes to prepare standard/sample dilutions
7. Plate cover
8. Absorbent filter papers
9. 100 ml and 1 L volume graduated cylinders

Protocol

A. Preparation of sample and reagents

1. Sample

Isolate the test samples soon after collecting and analyze immediately (within 2 hours) or aliquot and store at -20°C or -80°C for long term storage. Avoid multiple freeze-thaw cycles.

- **Serum:** Samples should be collected into a serum separator tube. Coagulate the serum at room temperature (~2 hr) or overnight at 4°C. Centrifuge at approximately 1000 × g for 20 min. Analyze the serum immediately or aliquot and store at -20°C or -80°C.
- **Urine:** Collect in a sterile container. Centrifuge at 10,000 × g for 2 min at 4°C.

Note:

» Samples must be diluted so that the expected concentration falls within the kit's range.

» Fresh samples or recently obtained samples are recommended to prevent protein degradation and denaturalization that may lead to erroneous results.

» Please bring sample slowly to room temperature. Sample hemolysis will influence the result. Hemolyzed specimen should not be used.

» NaN3 cannot be used as test sample preservative, since it inhibits HRP.

2. Wash buffer

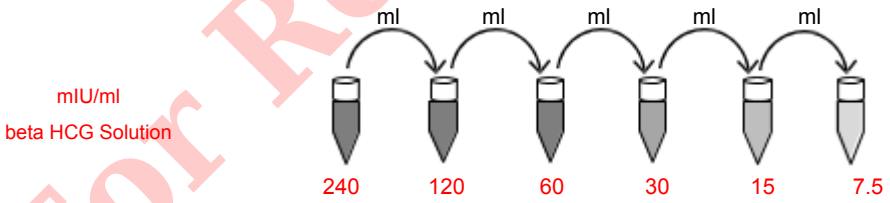
Dilute the concentrated Wash buffer 30-fold (1/30) with distilled water (i.e. add 20 ml of concentrated wash buffer into 580 ml of distilled water).

3. Standard

The standards should be prepared no more than 2 hours before the experiment. (Note; do not dilute standard directly in the plate).

1. 240 mIU/ml standard. Add µl of the mIU/ml standard into µl of the standard diluent buffer and mix thoroughly.

2. 120 mIU/ml → 7.5 mIU/ml standard solutions. Label 5 Eppendorf tubes with 120 mIU/ml, 60 mIU/ml, 30 mIU/ml, 15 mIU/ml and 7.5 mIU/ml. Aliquot µl of the Standard diluent buffer into each tube. Add µl of the above 240 mIU/ml standard solution into the first tube and mix thoroughly. Transfer µl from the first tube to the second tube and mix thoroughly, and so on.



B. Assay Procedure

Equilibrate the kit components and samples to room temperature prior to use. It is recommended to plot a standard curve for each test.

1. Set standard, test sample and control (zero) wells on the pre-coated plate respectively and record their positions.
2. Aliquot 50 µl of the diluted standards into the standard wells.
3. Aliquot 50 µl of Standard diluent buffer into the control (zero) well. Do not add sample and HRP Conjugate Reagent into the control (zero) well.
4. Aliquot 50 µl of appropriately diluted sample into the test sample wells. Add the solution at the bottom without touching the side walls of the well. Shake the plate mildly to mix the contents.
5. Seal the plate with a cover and incubate at 37°C for 30 min.

6. Remove the cover and wash the plate 5 times with one of the following methods.

Manual Washing: Discard the solution without touching the side walls. Tap the plate on absorbent filter papers or other absorbent material. Fill each well completely with Wash buffer and incubate on an ELISA shaker for 2 min. Discard the contents and tap the plate on absorbent filter papers or other absorbent material. Repeat this procedure three times.

Automated Washing: Discard the solution and wash the plate three times with Wash buffer (overfilling wells with the buffer). After the final wash, invert the plate and tap on absorbent filter papers or other absorbent material. It is recommended that the washer be set for a soaking time of 1 min.

7. Aliquot 50 µl of HRP Conjugate Reagent into each well (except control well).
8. Seal the plate with a cover and incubate at 37°C for 30 min.
9. Remove the cover and repeat the aspiration/wash process 5 times as explained in step 6.
10. Aliquot 50 µl of TMB Substrate A into each well and 50 µl of TMB Substrate B. Vortex gently the plate on ELISA shaker for 30 seconds (Or shake gently by hand for 30 seconds). Cover the plate and incubate at 37°C for 15 min. Avoid exposure to light.
11. Add 50 µl of Stop solution into each well. There should be a color change to yellow. Gently tap the plate to ensure thorough mixing.
12. Ensure that there are no fingerprints or water on the bottom of the plate, and that the fluid in the wells is free of bubbles. Measure the absorbance at 450nm immediately.

For calculation, (relative O.D.450) = (O.D.450 of each well) – (O.D.450 of Zero well). The standard curve can be plotted as the relative O.D.450 of each standard solution (Y) vs. the respective concentration of the standard solution (X). Log-log curve fitting is recommended for data analysis. The Human beta HCG concentration of the samples can be interpolated from the standard curve.

Note: If the samples measured were diluted, multiply the dilution factor by the concentrations from interpolation to obtain the concentration before dilution.

C. Precautions

1. Before using the kit, centrifuge the tubes to bring down the contents trapped in the lid.
2. Avoid foaming or bubbles when mixing or reconstituting components.
3. Wash buffer may crystallize and separate. If this happens, please warm the tube and mix gently to dissolve.
4. It is recommend to measure each standard and sample in duplicate or triplicate.
5. Do NOT let the plate dry out completely as this will inactivate the biological material on the plate.
6. Ensure plates are properly sealed or covered during incubation steps.
7. Complete removal of all solutions and buffers during wash steps is necessary for accurate measurement readings.
8. To avoid cross contamination do not reuse pipette tips and tubes.
9. Do not use expired components or components from a different kit.
10. Store the TMB substrate A and B in the dark and to avoid edge effect of plate incubation for temperature differences it is recommended to equilibrate the TMB substrates for 30 min at room temperature prior to use. Aspirate the dosage needed with sterilized tips and do not dump the residual solution back into the vial.

D. Typical Data & Standard Curve

Results of a typical standard run of a Human beta HCG ELISA Kit is shown below. This standard curve was generated at our lab for demonstration purpose only. Each user should obtain their own standard curve as per experiment.

Concentration mIU/ml	0	7.5	15	30	60	120	240
OD450	0.073	0.139	0.243	0.494	1.026	2.257