FREE BETA-SUBUNIT OF HUMAN CHORIONIC GONADOTROPIN (Free β-hCG) ENZYME IMMUNOASSAY TEST KIT
Catalog Number: OKBA00009

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Enzyme Immunoassay for the determination of Free Beta-Subunit of Human Chorionic Gonadotropin (Free β-hCG) in Human Serum

FOR Research Purposes Only

PRINCIPLE OF THE TEST

The free β-hCG ELISA test is based on the principle of a solid phase enzyme-linked immunosorbent assay.[1] The assay system utilizes a unique monoclonal antibody directed against a distinct antigenic determinant on the free β-hCG. Mouse monoclonal anti-free-β-hCG antibody is used for solid phase immobilization (on the microtiter wells). A goat whole hCG antibody is in the antibody-enzyme (horseradish peroxidase) conjugate solution. The test sample is allowed to react sequentially with the two antibodies, resulting in the free β-hCG molecules being sandwiched between the solid phase and enzyme-linked antibodies. After two separate 30 minute incubations at 37 °C, the wells are washed with water to remove unbound labeled antibodies. A solution of TMB Reagent is added and incubated for 20 minutes, resulting in the development of a blue color.

The color development is stopped with the addition of Stop Solution changing the color to yellow. The concentration of β-hCG is directly proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm.

REAGENTS

Materials provided with the kit:

- *Antibody-Coated Wells (1 plate, 96 wells)*
  Microtiter Wells coated with monoclonal anti-free-β-hCG
- *Reference Standard Set (1.0 ml/vial)*
  Contains 0, 2.5, 5, 10, 25, and 50 ng/ml of β-hCG in bovine serum with preservatives, lyophilized
- *Zero Buffer (13 ml)*
  Contains tris buffer with preservatives
- *Enzyme Conjugate Reagent (18 ml)*
  Contains goat anti-whole hCG conjugated to horseradish peroxidase with preservatives
- *TMB Reagent (11 ml)*
  Contains 3, 3', 5, 5' tetramethylbenzidine (TMB) stabilized in buffer solution
- *Stop Solution -1N HCl (11 ml)*
  Diluted hydrochloric acid

Materials required but not provided:

- Precision pipettes: 50 µl, 100 µl, 150 µl, and 1.0 ml.
- Disposable pipette tips.
- Distilled water.
- Vortex mixer or equivalent.
- Absorbent paper or paper towel.
- Graph paper.
- Microtiter plate reader.

WARNINGS AND PRECAUTIONS

1. CAUTION: This kit contains human material. The source material used for manufacture of this component tested negative for HBsAg, HIV 1/2 and HCV by FDA-approved methods. However, no method can completely assure absence of these agents. Therefore, all human blood products, including serum samples, should be considered potentially infectious. Handling should be as defined by an appropriate national biohazard safety guideline or regulation, where it exists.[2-4]
2. Avoid contact with 1N HCl. It may cause skin irritation and burns. If contact occurs, wash with copious amounts of water and seek medical attention if irritation persists.
3. Do not use reagents after expiration date and do not mix or use components from kits with different lot numbers.
4. Replace caps on reagents immediately. Do not switch caps.
5. Do not pipette reagents by mouth.
6. For Research Purposes Only.

STORAGE CONDITIONS

1. Store the unopened kit at 2-8°C upon receipt and when it is not in use, until the expiration shown on the kit label. Refer to the package label for the expiration date.
2. Keep microtiter plate in a sealed bag with desiccant to minimize exposure to damp air.

INSTRUMENTATION

A microtiter well reader with a bandwidth of 10 nm or less and an optical density range of 0 to 3 OD or greater at 450 nm wavelength is acceptable for absorbance measurement.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder.
2. Dispense 50 µl of standards, specimens, and controls into appropriate wells.
3. Dispense 100 µl of Zero Buffer into each well.
4. Thoroughly mix for 30 seconds. It is very important to mix them completely.
5. Incubate at 37 °C for 30 minutes.

6. Remove the incubation mixture by flicking plate contents into a sink.
7. Rinse and flick the microtiter wells 5 times with distilled or deionized water. (Please do not use tap water.)
8. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
9. Dispense 150 µl of Enzyme Conjugate Reagent into each well. Gently mix for 10 seconds.
10. Incubate at 37 °C for 30 minutes.
11. Remove the incubation mixture by flicking plate contents into a waste container.
12. Rinse and flick the microtiter wells 5 times with distilled or deionized water. (Please do not use tap water.)
13. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
14. Dispense 100 µl of TMB Reagent into each well. Gently mix for 10 seconds.
15. Incubate at room temperature for 20 minutes.
16. Stop the reaction by adding 100 µl of Stop Solution to each well.
17. Gently mix for 30 seconds. ***It is important to make sure that all the blue color changes to yellow color completely.***
18. Read optical density at 450 nm with a microtiter well reader ***within 15 minutes***.

QUALITY CONTROL

Good laboratory practice requires that quality control specimens (controls) be run with each calibration curve to verify assay performance. To ensure proper performance, control material should be assayed repeatedly to establish mean values and acceptable ranges.

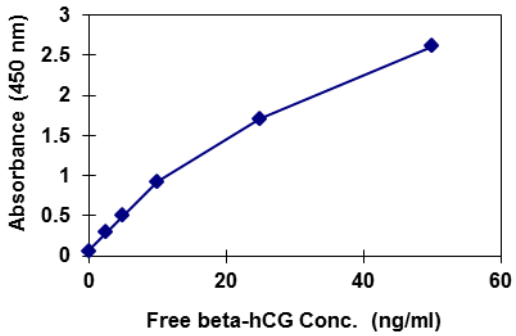
CALCULATION OF RESULTS

1. Calculate the mean absorbance value (A_{450}) for each set of reference standards, controls and samples.
2. Construct a standard curve by plotting the mean absorbance obtained from each reference standard against its concentration in ng/ml on graph paper, with absorbance values on the vertical or Y axis, and concentrations on the horizontal or X axis.
3. Use the mean absorbance values for each specimen to determine the corresponding concentration of free β-hCG in ng/ml from the standard curve.

EXAMPLE OF STANDARD CURVE

Results of a typical standard run with optical density readings at 450 nm shown in the Y axis against free β-hCG concentrations shown in the X axis. This standard curve is for the purpose of illustration only, and should not be used to calculate unknowns. Each user should obtain his or her own data and standard curve.

ß-hCG(ng/ml)	Absorbance (450 nm)
0	0.061
2.5	0.296
5.0	0.498
10.0	0.929
25.0	1.711
50.0	2.613



TECHNICAL CONSULTATION

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