FREE BETA-SUBUNIT OF CHORIONIC GONADOTROPIN (Free β-hCG) ENZYME IMMUNOASSAY TEST KIT
Catalog Number: EA100983

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

FOR RESEARCH USE ONLY

NOT FOR USE IN DIAGNOSTIC PROCEDURES

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 anti 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:

- Murine Monoclonal Anti-free-β-hCG-coated microtiter wells.
- Set of Reference Standards: 0, 2.5, 5, 10, 25, and 50 ng/ml, lyophilized.
- Zero Buffer (Sample diluent), 13 ml.
- Enzyme Conjugate Reagent, 18 ml.
- TMB Reagent (One-Step), 11 ml.
- Stop Solution (1N HCl), 11 ml.

STORAGE OF TEST KIT AND INSTRUMENTATION

Unopened test kits should be stored at 2-8 °C upon receipt and the microtiter plate should be kept in a sealed bag with desiccants to minimize exposure to damp air. Opened test kits will remain stable until the expiration date shown, provided it is stored as described above. A microtiter plate reader with a bandwidth of 10 nm or less and an optical density range of 0-2 OD or greater at 450 nm wavelength is acceptable for use in absorbance measurement.

REAGENT PREPARATION

1. All reagents should be brought to room temperature (18-25 °C) before use.
2. Reconstitute each lyophilized standard with 1.0 ml distilled water. Allow the reconstituted material to stand for at least 20 minutes and mix gently. Reconstituted standards will be stable for up to 30 days when stored sealed at 2-8 °C.

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***.

CALCULATION OF RESULTS

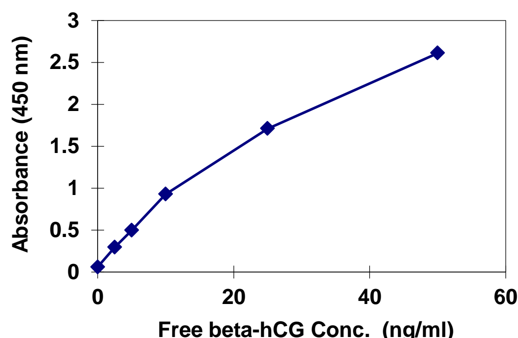
1. Calculate the mean absorbance value (A_{450}) for each set of reference standards, controls and patient samples.

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 (ng/ml) 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

OriGene Technologies, Inc.
9620 Medical Center Dr., Suite 200
Rockville, MD 20850

Phone: 1.888.267.4436
Fax: 301-340-9254
Email: techsupport@origene.com
Web: www.origene.com

091815

FOR RESEARCH USE ONLY

NOT FOR USE IN DIAGNOSTIC PROCEDURES