

TECO DIAGNOSTICS

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FREE β -HCG (MICROTITER ASSAY)

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

FOR IN VITRO DIAGNOSTIC USE ONLY

Store at 2 to 8°C.

PROPRIETARY AND COMMON NAMES

Free β -hCG Enzyme Immunoassay

INTENDED USE

For the quantitative determination of free beta subunit of human chorionic gonadotropin (free β -hCG) concentration in human serum.

INTRODUCTION

Human Chorionic Gonadotropin (hCG) is a glycoprotein hormone normally produced by placenta during pregnancy. The hormone is present in blood and urine around seven to thirteen days following implantation of the fertilized ovum. Structurally intact hCG molecules consist of two non-covalently linked polypeptide subunits, the alpha and beta chain subunits. Measurement of intact hCG and of the alpha subunit of hCG appears to give similar results in blood and urine but not the levels of beta subunit. In the normal second-trimester maternal sera, the level of intact hCG range from 20,000 mIU/ml to 50,000 mIU/ml. In contrast, the levels of either free α - or free β -hCG are on average one half of 1% of hCG levels. hCG and the free subunits appear not to be useful as serological markers for nontrophoblastic tumors; however, the absolute increase of β -hCG level in choriocarcinoma patients clearly differentiates it from normal pregnancy. Recent studies showed a significant increase in the level of free β -hCG subunit in trisomy 21 cases as compared with controls. Hence, it has been suggested that free β -hCG subunit assay in a combination of maternal serum AFP could be effective in a screening protocol for trisomy 21.

PRINCIPLE OF THE TEST

The free β -hCG ELISA test is based on the principle of a solid phase enzyme-linked immunosorbent assay.ⁱⁱⁱ The assay system utilizes a unique monoclonal antibody directed against a distinct antigenic determinant on the β -subunit of the hCG molecule. Mouse monoclonal anti- β -hCG antibody is used for solid phase immobilization (on the microtiter wells). A second monoclonal anti- β -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 β -hCG molecules being sandwiched between the solid phase and enzyme-linked antibodies. After incubation two separate 30 minute incubations at 37°C, the wells are washed with water to remove unbound labeled antibodies. A solution of H₂O₂/TMB is added and incubated for 20 minutes, resulting in the development of a blue color. The color development is stopped with the addition of 2N HCl 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 β -hCG-coated microtiter wells.
- Set of Reference Standards: 0, 2.5, 5, 10, 25, and 50 mIU/ml (WHO IRP 75/551), lyophilized.
- Zero Buffer (Sample diluent), 13ml.
- Enzyme Conjugate Reagent, 18 ml.
- Color Reagent A, 13 ml.
- Color Reagent B, 13 ml.
- Stop Solution (2N HCl), 10 ml.

Materials required but not provided:

- Precision pipettes: 50 μ l, 100 μ l, 150 μ l, and 1.0ml.
- Disposable pipette tips.
- Distilled water.
- Glass tubes or flasks to mix Color Reagents A and B.
- Vortex mixer or equivalent.
- Absorbent paper or paper towel.
- Graph paper.
- Microtiter plate reader.

SPECIMEN COLLECTION AND PREPARATION

Serum should be prepared from a whole blood specimen obtained by acceptable medical techniques. This kit is for use with serum samples without additives only.

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 10nm or less and an optical density range of 0-2 OD or greater at 450nm 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 should be stored sealed at 2-8°C
3. To prepare H₂O₂/TMB solution, make an 1:1 mixing of Color Reagent A with Color Reagent B up to 1 hour before use. Mix gently to ensure complete mixing. The prepared H₂O₂/TMB reagent should be made at least 15 minutes before use and is stable at room temperature in the dark for up to 3 hours. Discard excess after use.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder.
2. Dispense 50 μ l of standards, specimens, and controls into appropriate wells.
4. Dispense 100 μ l of Zero Buffer into each well.
4. Thoroughly mix for 30 sec. It is very important to mix completely.
5. Incubate at 37°C for 30 minutes.
6. Remove the incubation mixture by flicking contents into sink.
7. Rinse and flick the microtiter wells 5 times with distilled water.
8. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
9. Dispense 150 μ l of Enzyme Conjugate Reagent into each well. Gently mix for 5 seconds.
10. Incubate at 37°C for 30 minutes.
- **Prepare H₂O₂/TMB solution up to 15 minutes before use.**
11. Remove the incubation mixture by flicking plate contents into a waste container.
12. Rinse and flick the microtiter wells 5 times with running tap or distilled water.
13. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
14. Dispense 200 μ l of H₂O₂/TMB solution into each well. Gently mix for 5 seconds.
15. Incubate at room temperature for 20 minutes.
16. Stop the reaction by adding 50 μ l 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 450nm with a microtiter well reader within 30 minutes.

CALCULATION OF RESULTS

1. Calculate the mean absorbance value (A₄₅₀) 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 mIU/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 concentration of β -hCG in mIU/ml from the curve.

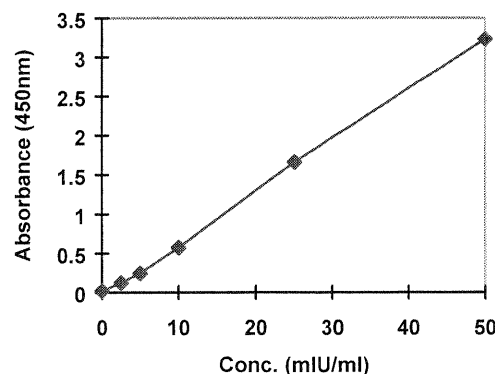
LIMITATIONS OF THE PROCEDURE

1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of package insert instructions and with adherence to good lab practice.
2. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
3. The results obtained from the use of this kit should be used only as an adjunct to other diagnostic procedures and information available to the physician.

EXAMPLE OF STANDARD CURVE

Results of a typical standard run with optical density readings at 450nm shown in the Y axis against 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.

β -hCG (mIU/ml)	Absorbance (450nm)
0	0.021
2.5	0.122
5.0	0.247
10.0	0.573
25.0	1.662
50.0	3.231



EXPECTED VALUES

Expected Values and Indications for Quantitative Free β -hCG Assay:

1. In early pregnancy, free β -hCG concentration was found 10-80 mIU/ml. The free β -hCG/intact hCG ratio was 3.08-3.28 percent. After 6 to 7 weeks the free β -hCG and the ratio value declined. During the second and third trimester, a constant ratio was observed about 1 percent.
2. Serum samples from 40 normal subjects were assayed, in this population, 99% of the values were less than 0.4 mIU/ml.
3. Serum hCG and free subunit levels in sera from patients with gestational choriocarcinoma were reported as follows:

From Ozuturk et al. Endocrinology, 1987

Patient Number	hCG (mIU/ml)	α -hCG (mIU/ml)	β -hCG (mIU/ml)
1	210,000	112	8,000
2	22,195	20	1,300
3	6,840	1	232
4	36,000	44	3,900
5	4,200	2	350

SENSITIVITY

The minimum detectable concentration of this β -hCG in this assay is estimated to be 0.5mIU/ml.

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