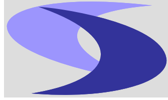


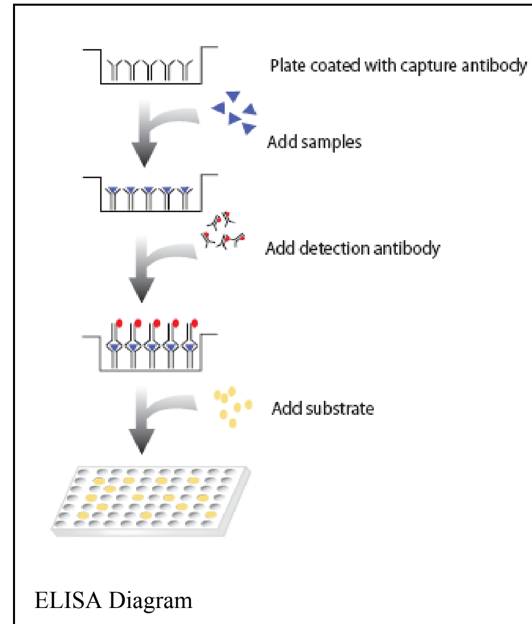
Signosis
BioSignal Capture

Free β-hCG ELISA

Catalog Number EA-0107 **(For Research Use Only)**

Introduction

Human Chorionic Gonadotropin (hCG) is a glycoprotein hormone normally produced by placenta during pregnancy (1). Structurally intact hCG molecules consist of two non-covalently linked polypeptide subunits, the alpha and beta chain subunits. Measurement of intact hCG and 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 (1 ng = 15 mIU). 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 (2-4).



ELISA Diagram

Principle of the assay

The free β-hCG ELISA is based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay system utilizes a anti-β-hCG antibody directed against β-hCG for solid phase immobilization (on the microtiter wells). A rabbit anti-β-hCG antibody conjugated to horseradish peroxidase (HRP) is in the antibody-enzyme conjugate solution. The test sample is allowed to react simultaneously with these antibodies, resulting in β-hCG being sandwiched between the solid phase and enzyme-linked antibodies. After incubation, the wells are washed to remove unbound-labeled antibodies. A HRP substrate, TMB, is added to result in the development of a blue color. The color development is then 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.

Materials provided with the kits:

- Monoclonal anti-β-hCG coated 96-well plate
- 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)
- Enzyme Conjugate Reagent (18 ml) Contains β-hCG MoAb conjugated to horseradish peroxidase
- TMB Reagent (11 ml)
- Stop Solution -1N HCl (11 ml)

Materials required but not provided:

- Microplate reader capable of measuring absorbance at 450 nm
- Deionized or distilled water.

Specimen Collection and Handling

Serum should be separated from the red blood cells as soon as possible. Specimens should be stored for up to 48 hours or -20°C for up to 6 months prior to assay. Avoid grossly hemolytic (bright red), lipemic (milky), or turbid samples (after centrifugation). Specimens should not be repeatedly frozen and thawed prior to testing. DO NOT store in "frost free" freezers, which may cause occasional thawing. Specimens which have been frozen, and those which are turbid and/or contain particulate matter, must be centrifuged prior to use.

Preparation for Assay

- All reagents should be taken to room temperature (18-25 ^{o}C) before use.
- 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 ^{o}C.

Assay Procedure

Samples of patient serum, plasma need to be diluted before use for best bet results

1. Add 50 µl of standard, specimens, and controls into appropriate wells.
2. Add 100 µl of Zero Buffer into each well.
3. Thoroughly mix for 30 seconds. It is very important to have a complete mixing in this setup.
4. Incubate at room temperature (18-25ºC) for 30 minutes.
5. Remove the incubation mixture by flicking plate content into a waste container.
6. Rinse and flick the microtiter wells 5 times with distilled or deionized water. Put the wells sharply onto paper towel to remove all residual water droplets.
7. Add 150 µl of Enzyme Conjugate Reagent into each well. Gently mix for 10 seconds.
8. Incubated at room temperature for 30 minutes.
9. Remove the incubation mixture by flicking plate contents into a waster container.
10. Rinse and flick the microtiter wells 5 times with distilled water
11. Tap the plate sharply onto paper towel to remove residual water droplets.
12. Dispense 100 µl TMB Reagent into each well, and mix gently for 10 seconds.
13. Incubate at room temperature in the dark for 20 minutes.
14. Add 100 µl Stop Solution to each well to stop the reaction.
15. Gently mix for 30 seconds. It is important to make sure that all the blue color changes to yellow color completely.
16. Read absorbance at 450nm with a microtiter well reader within 15 minutes.

References

1.Engall, E., Enzyme immunoassay ELISA and EMIT. In: Van Vunakis, H. and Langone, J.J. (eds.), *Methods in Enzymology*, Academic Press, New York, 1980; 70: 419-439.

2. Densem, J., and Wald, N.J., The stability of blood samples for the measurement of the free β subunit of chorionic gonadotrophin. Prenat. Diagn., 1995; 15: 94-95.

3. Ozturk, M., Berkowitz, R., Goldstein, D., Bellet, D., Wands, J. R., Differential production of human chorionic gonadotropin and free subunits in gestational trophoblastic disease. *Am. J. Obstet. Gynecol.*,1988; 158:193-198.

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Example of 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