Beta-Human Chorionic Gonadotropin (β-hCG)

ENZYME IMMUNOASSAY TEST KIT

Enzyme Linked Immunosorbent Assay (ELISA) for Quantitative Determination of Beta- Human Chorionic Gonadotropin (β-hCG) in Human Serum

FOR IN VITRO DIAGNOSTIC USE ONLY

Store at 2°C to 8°C

INTENDED USE

β-hCG Sandwich ELISA test for the quantitative determination of Beta-Human Chorionic Gonadotropin (β-hCG) in human serum.

INTRODUCTION

Human chorionic gonadotropin (hCG) is a sialoglycoprotein. HCG is initially secreted by the trophoblastic cells of the placenta shortly after implantation of the fertilized ovum into the uterine wall. The rapid rise in hCG serum levels after conception makes it an excellent marker for early confirmation and monitoring of pregnancy. Physiologically, hCG appears to maintain the corpus luteum, thereby allowing synthesis of progesterone and estrogens that support the endometrium. As uncomplicated pregnancies progress, the placenta assumes the production of these hormones. The serum hCG levels increase to a peak concentration, then decrease and plateau. HCG circulates as the intact molecule in the serum of normal women who have an uncomplicated pregnancy. The subunits are cleared rapidly and excreted by the kidney. The placental hormone, hCG, is similar to luteinizing hormone (LH), follicle stimulating hormone (FSH) and thyroid stimulating hormone (TSH). All are glycoproteins consisting of two non-covalently bound dissimilar subunits, designated alpha and beta, with attached carbohydrate sidechains. The alpha subunits of these glycoproteins are very similar. In contrast, the beta subunit portions determine the biological and immunochemical specificities. The beta subunits of hCG and LH exhibit considerable homology in amino acid content. Amino acid residues specific for the beta subunit of hCG confer the immuno-chemical specificity. With the availability of sensitive quantitative assays for the measurement of serum β-hCG, it has been shown that hCG levels can be useful in predicting spontaneous abortions, aiding in the detection of ectopic pregnancy and multiple gestation. Elevated levels of hCG have also been detected in serum from patients with abnormal physiological conditions not related to pregnancy.

PRINCIPLE OF THE ASSAY

β-hCG Quantitative Test Kit is a sandwich-based enzyme-linked immunosorbent assay. The test employs one anti- β-hCG antibody for solid phase (microtiter wells) immobilization and another mouse monoclonal anti β-hCG antibody in the antibody-enzyme (horseradish peroxidase) conjugate solution. The test sample is added to the hCG antibody coated microtiter wells and incubated with the Zero Buffer. If antigen is present in the specimen, it will combine with the antibody on the well. The well is then washed to remove any residual test specimen, and hCG antibody labeled with horseradish peroxidase (conjugate) is added. The conjugate will bind immunologically to the β-hCG on the well, resulting in the antigen molecules being sandwiched between the solid phase and enzyme-linked antibodies. After incubation, the wells are washed to remove unbound labeled antibodies. A solution of TMB is added resulting in the development of a blue color. The color development is stopped by addition of stop solution and absorbance is determined for each well using an ELISA reader. The concentration of antigen is directly proportional to the color intensity of the test sample.

MATERIALS AND COMPONENTS

Materials provided with the test kits:

- Coated Microwells: Microwells coated with anti- hCG antibody.
- β-hCG Zero buffer. Ready to use
- β-hCG Enzyme Conjugate. Ready to use.
- TMB Substrate
- Stop Solution
- β-hCG standard set of 6 standards labelled as A to F in liquid form. For standard concentrations refer vial label.
- Wash Buffer Concentrate (20X)
- Control Set
- Pack Insert
- Plate Sealer
- Protocol Sheet
- Microwell Holder

Materials required but not provided

- Precision pipettes: 10-100µl, 20-200µl, 100-1000µl
- Disposable pipette tips
- Distilled water
- Disposable Gloves
- ELISA reader
- ELISA washer

STORAGE AND STABILITY

1. **β-hCG** kit is stable at 2-8°C upto expiry date printed on the label.
2. Coated microwells should be used within one month upon opening the pouch provided that once opened, the pouch must be resealed to protect from moisture. If the colour of the dessicant has changed from blue to pink at the time of opening the pouch, another coated microwells pouch should be used.
3. Diluted Wash Buffer is stable for upto one week when stored at 2-8°C.

SPECIMEN COLLECTION

1. Collect Blood specimen by venipuncture according to the standard procedure.
2. Only serum should be used.
3. Avoid grossly hemolytic, lipemic or turbid samples.
4. Preferably use fresh samples. However, specimens can be stored up to 48 hours at 2-8°C, for short duration.
5. For longer storage, specimens can be frozen at -20°C. Thawed samples must be mixed prior to testing.
6. Do not heat inactivate before use.
7. Specimen containing precipitate or particulate matter should be clarified by centrifugation prior to use.
8. Specimen should be free from particulate matter and microbial contamination.

PRECAUTIONS

1. Bring all reagents and specimen to room temperature before use.
2. Do not pipette any material by mouth.
3. Do not eat, drink or smoke in the area where testing is done.
4. Use protective clothing and wear gloves when handling samples.
5. Use absorbent sheet to cover the working area.
6. Immediately clean up any spills with sodium hypochlorite.
7. All specimens, standards and controls should be considered potentially infectious and discarded appropriately.
8. Neutralize acid containing waste before adding hypochlorite.
9. Do not use kit after the expiry date.
10. Do not mix components of one kit with another.
11. Always use new tip for each specimen and reagent.
12. Do not allow liquid from one well to mix with other wells.
13. Do not let the strips dry in between the steps.

REAGENT PREPARATION

1. All reagents should be brought to room temperature (18-25°C) and mixed by gently inverting or swirling prior to use. Do not induce foaming.
2. Dilute Wash Buffer 20 times (for example add 5 ml concentrated buffer to 95 ml distilled or deionized water). Mix well before use.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder. Dispense **25 µl** of Standards, controls and sera into the appropriate wells.
2. Dispense **50 µl** of Zero Buffer into each well. Incubate at room temperature (18-25°C), for **20 minutes**.
3. After incubation, empty the microtitre wells and wash the plate **5 times** with **350 µl** of diluted wash buffer. Strike the microtitre plate sharply onto the absorbent paper towel to remove all residual droplets.
4. Dispense **75 µl** of Enzyme Conjugate into each well. Incubate at room temperature (18-25°C) in the dark, for **20 minutes**.
5. After incubation, empty the microtitre wells and wash the plate **5 times** with **350 µl** of diluted wash buffer. Strike the microtitre plate sharply onto the absorbent paper towel to remove all residual droplets.
6. Dispense **100 µl** of TMB Substrate into each well. Incubate at room temperature (18-25°C), in the dark, for **20 minutes**.
7. Stop the reaction by adding **100 µl** of Stop Solution to each well. Gently mix for 10 seconds until the blue color completely changes to yellow.
8. Read the optical density at 450/630 nm with a microtiter plate reader within **15 minutes**.

Add **25 µl** Standards/controls/samples into the respective Microwells

⬇

Add **50 µl** Zero Buffer in each well

⬇

Apply plate sealer and incubate for **20 minutes** at **18-25°C**

⬇

Wash Microwells **5 times** with **350 µl** of diluted wash buffer

⬇

Add **75 µl** Enzyme conjugate in each well

⬇

Apply plate sealer and incubate for **20 minutes** at **18-25°C**

⬇

Wash Microwells **5 times** with **350 µl** of diluted wash buffer

⬇

Add **100 µl** substrate in each well

⬇

Incubate for **20 minutes** at **18-25°C in dark**

⬇

Add **100 µl** stop solution in each well

⬇

Read results @450nm (Ref.600-700nm) within **15 minutes**

CALCULATION OF RESULTS

Construct a standard curve by plotting the absorbance obtained from each reference standards against its concentrations in mIU/ml on the graph paper, with absorbance values on the vertical or Y axis and concentrations on the horizontal or X axis. Use the absorbance values for each specimen to

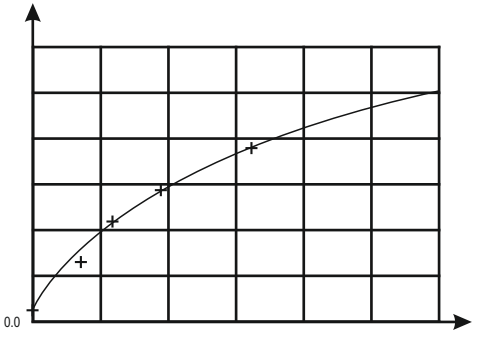
determine the corresponding concentration of Total β-hCG in mIU/ml from the standard curve. Any diluted specimens must be corrected by the appropriate dilution factor.

Example of Standard curve

Results of a typical Standard run with optical density reading at 450nm (ref 600-700nm) shown in the Y axis against β-hCG concentrations shown in the X axis.

Suggest: Use 4-Parameter Standard curve to calculate sample values.

Total β-hCG (mIU/ml)	Absorbance (450nm)
A	0.046
B	0.132
C	0.256
D	0.593
E	1.414
F	2.015



This standard curve is for the purpose of illustration only and should not be used to calculate samples. Each user should obtain his or her own standard curve and data.

Expected values and sensitivity

Each laboratory must establish its own normal ranges based on patient population. hCG is not normally detected in the serum of healthy men or healthy non-pregnant women. The concentration of hCG in the serum of pregnant women increases to 5-50 mIU/ml one week after implantation and continues increasing exponentially during the first ten weeks, reaching a maximum of 100,000-200,000 mIU/ml at the end of the first trimester. The minimum detectable concentration of hCG by this assay is estimated to be 2.0 mIU/ml.

PERFORMANCE CHARACTERISTICS

A) Internal Evaluation:

1. Accuracy: In an internal study **β-hCG** was evaluated against commercially available licensed kit with 90 random clinical samples & **β-hCG** has demonstrated 100 % clinical correlation with the commercially available licensed kit.

   Precision: **β-hCG** was evaluated with licensed external Quality controls for Precision Studies & following is the data:

Controls	No. of testings	Mean Control values with **β-hCG**	Coefficient of Variation (CV)
Level 1	10	1.70	7.25
Level 2	10	8.26	5.72
Level 3	10	72.00	4.93

B) External Evaluation:

β-hCG ELISA has been evaluated by a NABL accredited lab against their reference method. In this evaluation **β-hCG** ELISA has demonstrated 98% correlation with the reference method.

*Data file: Orchid Biomedical Systems (P) Ltd.

IMPORTANT NOTE

1. This assay is temperature sensitive assay. The best temperature condition for this assay is from 18°C to 25°C.
2. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
3. It is recommended to use the multi channel pipettes to avoid time effect. A full plate of 96 wells may be used if automated pipetting is available.
4. Duplication of standards, samples and controls is not mandatory but may provide information on reproducibility & application errors.

LIMITATIONS OF THE ASSAY

(1). As with all diagnostic tests, a definite clinical diagnosis should not be based on the results of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated. (2). The activity of the enzyme used is temperature-dependent and the OD values may vary. The higher the room temperature (+18°C to +25°C) during substrate incubation, the greater will be the OD values. Corresponding variations apply also to the incubation times. However, the standards are subject to the same influences, with the result that such variations will be largely compensated in the calculation of the result. (3). Adaptation of this assay for use with automated sample processors and other liquid handling devices, in whole or in part, may yield differences in test results from those obtained using the manual procedure. It is the responsibility of each laboratory to validate that their automated procedure yields test results within acceptable limits. (4).Insufficient washing (e.g., less than 5 wash cycles, too small wash buffer volumes, or shortened reaction times) can lead to incorrect OD values.

BIBLIOGRAPHY

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6. Data on file: Orchid Biomedical Systems (P) Ltd.

SYMBOL KEYS

Temperature Limitation		Consult Instructions for use	
Manufacturer		IVD	*In vitro* Diagnostic Medical Device
Use by		REF	Catalogue Number
Date of Manufacture		LOT	Batch Number / Lot Number
This side up		Σ	Contains sufficient for <n> tests
Do not reuse			

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