



Beta- HUMAN CHORIONIC GONADOTROPIN (-HCG) ENZYME

IMMUNOASSAY TEST KIT
Cat no. 10010

Enzyme Immunoassay for the Quantitative Determination of Beta-Human Chorionic Gonadotropin in Human Serum (For In Vitro Diagnostic Use Only)

Intended use

β-hCG EIA test kit is mainly intended to quantitatively determine Total beta-hCG concentration in human serum.

Introduction

Human chorionic gonadotropin (hCG) is a sialoglycoprotein with a molecular weight of approximately 46,000 daltons. 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 human thyroid stimulating hormone (hTSH). All are glycoproteins consisting of two noncovalently 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 immunochemical specificity.

With the availability of sensitive quantitative assays for the measurement of serum -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.

The hCG EIA test provides a rapid, sensitive and reliable assay. The

Principle of the test

The hCG Quantitative Test Kit is based on a solid phase enzyme-linked immunosorbent assay. The assay system utilizes one anti-hCG antibody for solid phase (microtiter wells) immobilization and another mouse monoclonal anti-βhCG antibody in the antibody-enzyme (horseradish peroxidase) conjugate solution. The test specimen (serum) is added to the hCG antibody coated microtiterwells 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) are 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 on incubation at room temperature, the wells are washed with water to remove unbound labeled antibodies. A solution of 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, and the color is changed to yellow and measured spectrophotometrically at 450 nm. The concentration of antigen is directly proportional to the color intensity of the test sample.

Materials and components

Materials provided with the test kits:

- Antibody-coated microtiter wells.
- Reference standards, 0,5,20,50,150,300 mIU/mL, liquid standard, ready for use.
- Zero buffer, 12 mL
- Enzyme Conjugate Reagent, 18 mL
- TMB Substrate, 12 mL
- Stop Solution, 12 mL.
- Wash Buffer Concentrate(50X), 15 mL

Materials required but not provided:

- Precision pipettes: 0.04~0.2mL, 1.0 mL
- Disposable pipette tips
- Distilled water
- Vortex mixer or equivalent
- Absorbent paper or paper towel
- Semi-log graph paper
- Microtiter well 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 kits 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. Unopened test kits will remain stable until the expiring date shown, provided it is stored as prescribed above. A microtiter plate reader with a bandwidth of 1nm or less and an optical density range of 0-3 OD or greater at 450nm wavelength is acceptable for use in absorbance measurement.

Reagent preparation

- 1.All reagent should be brought to room temperature (18-22°C) before use.
- 2.Dilute 1 volume of Wash Buffer (50x) with 49 volumes of Distilled water. For example, Dilute 15 mL of Wash Buffer Concentrate (50x) into distilled water to prepare 750mL of washing buffer(1x). Mix well before use.

Assay procedures

- 1.Secure the desired number of coated wells in the holder.
- 2.Dispense 50µL of standard,specimens, and controls into appropriate wells.
- 3.Dispense 100µL of hCG Zero Buffer into each well.
- 4.Thoroughly mix for 10 seconds. It is very important to have complete mixing in this setup.
- 5.Incubate at room temperature (18-22°C) for 30 minutes.
- 6.Remove the incubation mixture by flicking plate content into a sink.
- 7.Rinse and flick the microtiter wells 5 times with washing buffer(1X).
- 8.Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
- 9.Dispense 150µL of Enzyme Conjugate Reagent into each Well.Gently mix for 5 seconds.
- 10.Incubate at room temperature for 30 minutes.
- 11.Remove the incubation mixture by flicking plate contents into sink.
- 12.Rinse and flick the microtiter wells 5 times with washing buffer(1X).
- 13.Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
- 14.Dispense 100µL TMB substrate into each well. Gently mix For 5 seconds.
- 15.Incubate at room temperature in the dark for 20 minutes.
- 16.Stop the reaction by adding 100µL of stop solution to each well.
- 17.Gently mix for 5~30 seconds. It is very important to make sure that the blue color changes to yellow color completely.
- 18.Read optical density at 450nm with a microtiter plate Reader Within 30 minutes.

Important Note

The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance reading.

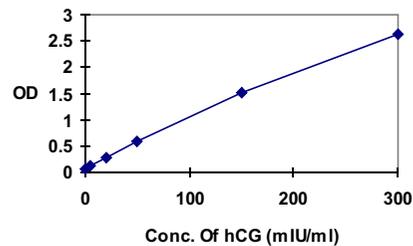
Calculation of results

Calculate the mean absorbance value (A450) for each set of reference standards, specimens, controls and patient samples. Construct a standard curve by plotting the mean absorbance obtained from each reference standard against its concentration in mIU/mL on semi-log graph paper, with absorbance values on the vertical or Y axis and concentrations on the horizontal or X axis. Use the mean absorbance values for each specimen to determine the corresponding concentration of total β-hCG in mIU/mL from the standard curve.

Example of standard curve

Results of typical standard run with optical density reading at 450nm shown in the Y axis against hCG concentrations shown in the X axis.

Total -hCG (mIU/mL)	Absorbance (450nm)
0	0.063
5	0.120
20	0.269
50	0.581
150	1.503
300	2.624



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.

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

1. Accuracy: Comparison between our kits and commercial available Kits provides the following data

N = 122
 Correlation Coefficient = 0.988
 Slope = 0.930
 Intercept = 1.08
 Mean (Our kits) = 24.3
 Mean (Abbott's Kits) = 25.0

2. Precision

1) Intra-Assay:

Concentrations	Replicates	Mean	S.D.	% CV
Level I	20	16.27	0.970	5.96
Level II	20	86.26	4.290	4.97

2]. Inter-Assay:

Concentrations	Replicates	Mean	S.D.	% CV
Level I	20	6.32	0.624	9.87
Level II	20	22.98	1.583	6.89
Level III	20	194.39	9.875	5.08

3. Linearity

Two patient sera were serially diluted with 0 mIU/mL standard in a linearity study. The average recovery was 102.9 %.

Sample A			
Dilution	Expected	Observed	% Recov.
Undiluted	210.0	210.0	
2x	105.0	107.8	102.7%
4x	52.5	54.8	104.4%
8x	26.3	25.9	98.6%
16x	13.1	14.1	107.5%
Average Recovery: 103.3 %			

Sample B			
Dilution	Expected	Observed	% Recov.
Undiluted	168.20	168.20	
2x	84.10	83.11	98.8%
4x	42.05	40.98	97.5%
8x	21.03	22.34	106.3%
16x	10.51	11.29	107.4%
Average Recovery: 102.5 %			

4. Recovery

Various patient serum samples of known hCG levels were mixed and assayed in duplicate. The average recovery was 101.9 %.

Expected Concentration	Observed Concentration	% Recovery
92.70	94.20	101.6
77.63	79.31	102.2
23.24	23.10	99.4
156.18	159.23	102.0
206.06	210.10	102.0
148.94	150.52	101.1
149.73	148.97	99.50
Average Recovery: 101.1 %		

5. Sensitivity

The minimum detectable concentration of this assay is estimated to be 2.0 mIU/mL.

6. Cross-reactivity

The following human materials were tested for crossreactivity of the assay:

Antigens	Concentration	Equivalent AFP	% Cross-reactivity
HCG	400 IU/mL	0.00	0.00
PAP	1,000 ng/mL	0.00	0.00
PSA	1,000 ng/mL	0.00	0.00
CEA	1,000 ng/mL	0.00	0.00

7. Hook Effect

No hook effect was observed in this assay.

Limitations of the Procedure

There are some limitation of the assay. We should let our customers know about that.

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) Studies have implicated possible interference in immunoassay results in some patients with known rheumatoid factor and antinuclear antibodies. Serum samples from patients who have received infusions containing mouse monoclonal antibodies for diagnostic or therapeutic purposes, may contain antibody to mouse protein (HAMA). Although we have added some agents to avoid the interferences, we cannot guarantee to eliminate all the effects of that.

3) The wash procedure (steps 6-8) is critical. Insufficient washing will result in poor precision and falsely elevated absorbance. The use of tap water for washing could result in a higher background absorbance.

References

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