AccuDiag™
Beta-Human Chorionic Gonadotropin (β- hCG) total ELISA Test Kit

REF 4201-16

IVD See External Label 2°C 8°C Σ 96 Tests

β- hCG ELISA	
Method	Enzyme Linked Immunosorbent Assay
Principle	Sandwich Complex
Detection Range	0-300 mIU/mL
Sample	50 µL
Sensitivity	2 mIU/mL
Incubation Time	80 minutes
Shelf Life	12 Months from the manufacturing date

PRODUCT FEATURES

- Very easy to use with little training
- Highly specific and consistent Assay
- Provides accurate results quickly
- Reading of results both visually and as absorbance data

INTENDED USE

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

SIGNIFICANCE AND SUMMARY

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 hommone (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 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.

The hCG EIA test provides a rapid, sensitive and reliable assay. The antibodies developed for the test will determine a minimal concentration of 2 mIU/ml.

ASSAY PRINCIPLE

β-hCG ELISA 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 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) 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 an 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.

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.

REAGENTS

Materials provided with the test kit

1. Antibody-coated microtiter wells.
2. Reference standards, 0, 5, 20, 50,150,300 mIU/ml, liquid standard, ready for use.
3. Zero buffer, 12 ml
4. Enzyme Conjugate Reagent, 18 ml
5. TMB Substrate,12 ml
6. Stop Solution, 12 ml.
7. Wash Buffer Concentrate(50X),15 ml
8. Control set (Optional)

Materials required but not provided

1. Precision pipettes: 0.04~0.2ml,1.0 ml
2. Disposable pipette tips
3. Distilled water
4. Vortex mixer or equivalent
5. Absorbent paper or paper towel
6. Semi-log graph paper
7. Microtiter well reader

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 PROCEDURE

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 step.
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 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 15 minutes.

Important Note:

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

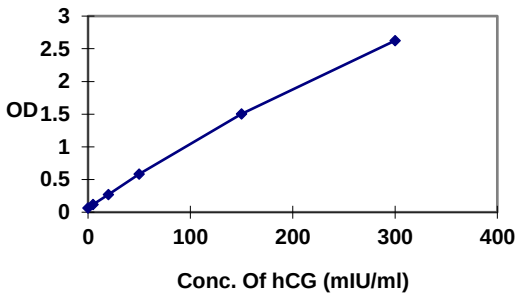
RESULTS

Calculate the mean absorbance value (A_{450}) 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. 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.

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



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 three weeks after last menstrual period (LMP) 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. Based on literature, expected hCG levels in pregnant women were shown in the table below. These ranges should be used as guidelines only. Each laboratory should establish its own reference ranges.

The minimum detectable concentration of hCG by this assay is estimated to be 2.0 mIU/ml.

Weeks since LMP	hCG level (mIU/ml)
3	5 - 50
4	5 - 426
5	18 - 7,340
6	1,080 - 56,500
7-8	7,650 - 229,000
9-12	25,700 - 288,000
13-16	13,300 - 254,000
17-24	4,060 - 165,400
25-40	3,640 – 117,000

STORAGE

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 0-2.5 OD or greater at 450nm wavelength is acceptable for use in absorbance measurement.

LIMITATIONS

There are some 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. 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 it eliminate all the effects of that.

REFERENCES

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MANUFACTURER AND BRAND DETAILS

ISO 13485:2016

ISO 13485 Quality Management for Medical Devices CERTIFIED

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Date Adopted	**2022-09**
REF **4201-16**	**AccuDiag™ - ß-hCG (Total) ELISA**
Brand Name	**AccuDiag™**
EC REP	**CEpartner4U, Esdoornlaan 13, 3951 DB Maarn, The Netherlands.** www.cepartner4u.eu
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