



HUMAN CHORIONIC GONADOTROPIN (hCG) ENZYME IMMUNOASSAY TEST KIT

Enzyme Immunoassay for the quantitative determination of human Chorionic Gonadotropin (hCG) concentration in human serum

For In-Vitro and professional use only

Store at 2 to 8°C.

INTRODUCTION

Human chorionic gonadotropin (hCG) is a glycoprotein hormone normally produced by the placenta during pregnancy. The hCG molecule consists of two combined, dissimilar subunits designated as alpha and beta. The beta subunit, with a molecular weight of approximately 30,000 Daltons, confers biological and immunological specificity to the entire hCG molecule by virtue of its unique amino acid sequence and content. The alpha subunit, with a molecular weight of approximately 18,000 Daltons, is essentially identical to the alpha subunit of the pituitary glycoprotein hormones: Luteinizing hormone (LH), follicle stimulating hormone (FSH), and thyroid stimulating hormone (TSH).

The appearance of hCG in urine or serum soon after conception and its rapid rise in concentration makes it an ideal indicator for the detection and confirmation of pregnancy. However, elevated hCG levels are also frequently associated with trophoblastic and non-trophoblastic neoplasms; these conditions should be considered before a diagnosis of pregnancy can be made.

Immunoassays utilizing antibodies specific to the beta subunit of hCG provide a sensitive and specific technique allowing early detection of pregnancy around the time of the first missed menstrual period.

In women with a multiple pregnancy (twins, triplets, etc.), levels of hCG have been reported to be higher than those expected during a normal single pregnancy. This is probably a result of the increased placental mass necessary to sustain multiple fetuses. Also, as one might suspect, cases of placental insufficiency show levels of hCG lower than expected during normal pregnancy. Decreased values have also been associated with threatened abortion and ectopic pregnancy.

PRINCIPLE OF THE TEST

The hCG quantitative test is based on a solid phase enzyme-linked immunosorbent assay (ELISA). The assay system utilizes a mouse monoclonal anti- α -hCG antibody for solid phase (microtiter wells) immobilization and a mouse monoclonal anti- β -hCG antibody in the antibody-enzyme (horseradish peroxidase) conjugate solution. The test specimen (serum) is added to the α -hCG antibody coated microtiter wells and incubated with the Zero buffer at room temperature for 30 minutes. If hCG is present in the specimen, it will combine with the antibody on the wells. The wells are then washed to remove any residual test specimen, and β -hCG monoclonal antibody labeled with horseradish peroxidase (conjugate) is added. The conjugate will bind immunologically to the hCG on the wells, resulting in the hCG molecule being sandwiched between the solid phase and enzyme linked antibodies. After incubation at room temperature for 15 minutes, the wells are washed with water to remove unbound labeled antibodies. A solution of TMB reagent is added and incubated at room temperature for 20 minutes, resulting in the development of a blue color. The color development is stopped with the addition of stop solution, and the color is changed to yellow and measured spectrophotometrically at 450nm. The concentration of hCG is directly proportional to the color intensity of the test sample.

REAGENTS

Materials provided with the kit:

1. Mouse monoclonal anti- α -hCG coated microtiter plate, 96 wells
2. Zero buffer, 13ml
3. Enzyme conjugate reagent, 18ml
4. hCG reference standards, containing 0, 5, 20, 50, 150 and 300 mIU/ml of hCG. Liquid. 1 set. Ready for use.
5. TMB reagent, 11ml
6. Stop solution (1N HCl), 11ml

Materials required but not provided with the kit:

1. Precision pipettes, 50 μ l, 100 μ l, and 150 μ l
2. Distilled water
3. Disposable pipette tips
4. Vortex mixer or equivalent
5. Absorbent paper or paper towel

- A microtiter plate reader at 450nm wavelength, with a bandwidth of 10nm or less and an optical density range of 0-2 OD or greater.
- Graph paper

STORAGE OF TEST KITS

- Store the kit at 2-8°C.
- Keep microwells sealed in a dry bag with desiccants.
- The reagents are stable until expiration date of the kit.
- Do not expose test reagents to heat, sun or strong light during storage or usage.
- Amicrotiter plate reader with a bandwidth of 10nm or less and an optical density range of 0-2 OD or greater at 450 nm wavelength is acceptable for use in absorbance measurement.

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.

REAGENT PREPARATION

All reagents should be allowed to reach room temperature (18-25°C) before use.

ASSAY PROCEDURE

- Place the desired number of coated wells into the holder.
 - Dispense 50µl of standards, specimens, and controls into the appropriate wells.
 - Dispense 100µl of zero buffer in each well.
 - Thoroughly mix for 30 seconds. It is very important to have a complete mixing in this step.
 - Incubate at room temperature (18-25°C) for 30 minutes.
 - Remove the incubation mixture by flicking plate content into sink
 - Rinse and flick the microtiter plate 5 times with distilled water or de-ionized water. (Do not use tap water)
 - Strike the microtiter plate sharply onto absorbent paper or paper towel to remove all residual water droplets
 - Dispense 150 µl of enzyme conjugate reagent into each well. Gently mix for 10 seconds.
 - Incubate at room temperature for 15 minutes.
 - Remove the incubation mixture by flicking plate contents into sink
 - Rinse and flick the microtiter wells 5 times with distilled water or de-ionized water. (Do not use tap water).
 - Strike the microtiter plate sharply onto absorbent paper or paper towel to remove all residual water droplets
 - Dispense 100 µl TMB reagent into each well. Gently mix for 10 seconds.
 - Incubate at room temperature in the dark for 20 minutes.
 - Stop the reaction by adding 100 µl of stop solution to each well.
 - Gently mix for 30 seconds. It is important to make sure that all the blue color changes to yellow color completely.
- Note: Make sure there is no air bubbles in each well before reading.**
- Read O.D. at 450 nm *within 15 minutes* with a microwell reader.

CALCULATION OF RESULTS

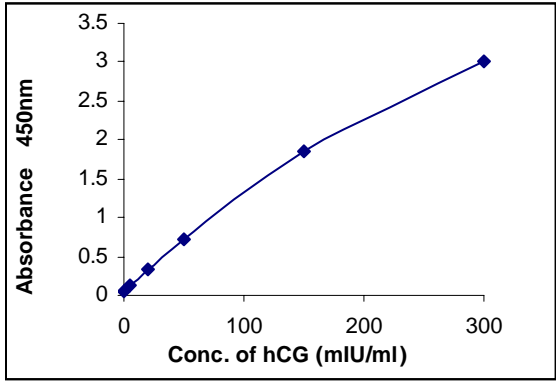
- Calculate the mean absorbance value (A450) for each set of reference standards, specimens, controls and patient samples
- Construct a standard curve by plotting the mean absorbance obtained from each reference standard against its concentration in mIU/ml on linear graph paper, with absorbance values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

Example:

Results of a typical standard run with optical density readings 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 is each experiment.

hCG Values (mIU/ml)	Absorbance (450nm)
0	0.051
5	0.134
20	0.328
50	0.724
150	1.852

300	3.011
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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 5-50mIU/ml one week after implantation and continues increasing exponentially during the first ten weeks, reaching a maximum of 250,000 – 50,000 mIU/ml at the end of the first trimester. The minimum detectable concentration of hCG by this assay is estimated to be 2.0mIU/ml

LIMITATIONS OF THE PROCEDURE

1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the package insert instructions and with adherence to good laboratory practice.
2. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
3. Serum samples demonstrating gross lipemia, gross hemolysis, or turbidity should not be used with this test.
4. 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.

REFERENCES

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ATLAS Medical
William James House,
Cowley Road, Cambridge, CB4 4WX, UK
Tel: ++44 (0) 1223 858 910
Fax: ++44 (0) 1223 858 524

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