Human Chorionic Gonadotropin (hCG) ELISA Kit Protocol

(Cat. No.:EK-310-27)

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INTENDED USE

For the quantitative determination of Human Chorionic Gonadotropin (hCG) concentration in human serum. **FOR RESEARCH ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES**

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.

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INTRODUCTION AND PROTOCOL OVERVIEW

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 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 neoplasmas; 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 the 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 those expected during normal pregnancy. Decreased values have also been associated with threatened abortion and ectopic pregnancy.

CAUTION: Phoenix Pharmaceuticals guarantees that its products conform to the information contained in this publication. The purchaser must determine the suitability of the product for its particular use and establish optimum sample concentrations.

PRINCIPLE OF THE TEST

The hCG Quantitative Test is based on a solid phase enzyme-linked immunosorbent assays (ELISA). The assay system utilizes a mouse monoclonal anti-alpha-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 alpha-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 molecules 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 450 nm. The concentration of hCG is directly proportional to the color intensity of the test sample.

LIST OF COMPONENTS

Materials Provided with the Kit:

- Mouse monoclonal anti-alpha-hCG coated microtiter plate, 96 wells.
- Zero Buffer, 13 ml.
- Enzyme Conjugate Reagent, 18 ml.
- hCG reference standards, containing 0, 5, 20, 50, 150, and 300 mIU/ml (WHO, 1st IRP/3rd IS 75/537). Liquid, 1 set. Ready for use.
- TMB Reagent, 11 ml.
- Stop Solution (1N HCl), 11 ml.

Materials required but not provided:

- Precision pipettes, 50 µl, 100 µl and 150 µl.
- Distilled water.
- Disposable pipette tips.
- Vortex mixer or equivalent.
- Absorbent paper or paper towel.
- A microtiter plate reader at 450 nm wavelength, with a bandwidth of 10 nm or less and an optical density range of 0-2 OD or greater.
- Graph paper.

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

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. Opened test kits will remain stable until the expiration date shown, provided it is stored as described above. A microtiter plate reader with a bandwidth of 10 nm or less and an optical density range of 0-2 OD or greater at 450 nm wavelength is acceptable for use in absorbance measurement. DO NOT FREEZE

REAGENT PREPARATION

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

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder.
2. Dispense 50 µl of standard, specimens, and controls into appropriate wells.
3. Dispense 100 µl of Zero Buffer into each well.
4. Thoroughly mix for 30 seconds. It is very important to have a complete mixing in this setup.
5. Incubate at room temperature (18-25°C) for 30 minutes.
6. Remove the incubation mixture by flicking plate content into a waste

container.

7. Rinse and flick the microtiter plate 5 times with distilled or deionized water. (Please do not use tap water.)
8. Strike the microtiter plate sharply onto absorbent paper or paper towels to remove all residual water droplets.
9. Dispense 150 µl of Enzyme Conjugate Reagent into each well. Gently mix for 10 seconds.
10. Incubate at room temperature for 15 minutes.
11. Remove the incubation mixture by flicking plate contents into sink.
12. Rinse and flick the microtiter wells 5 times with distilled or deionized water. (Please do not use tap water.)
13. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
14. Dispense 100 µl TMB Reagent into each well. Gently mix for 10 seconds.
15. Incubate at room temperature in the dark for 20 minutes.
16. Stop the reaction by adding 100 µl of Stop Solution to each well.
17. Gently mix for 30 seconds. It is important to make sure that all the blue color changes to yellow color completely.
18. Read the optical density at 450 nm with a microtiter plate reader within 15 minutes.

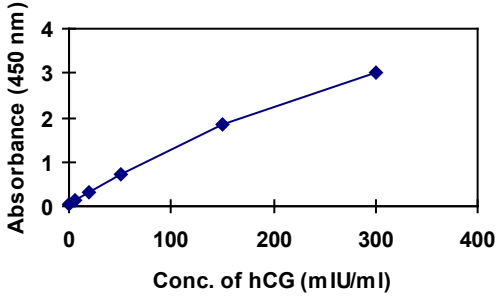
CALCULATION OF RESULTS

1. Calculate the mean absorbance value (A450) for each set of reference standards, specimens, controls and patient samples.
2. 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.
3. Using the mean absorbance value for each sample, determine the corresponding concentration of hCG in mIU/ml from the standard curve.

EXAMPLE OF STANDARD CURVE

Results of a typical standard run with optical density readings at 450 nm 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 in each experiment.

hCG Values (mIU/ml)	Absorbance (450 nm)
0	0.051
5	0.134
20	0.328
50	0.724
150	1.852
300	3.011



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 250,000-500,000 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.

REFERENCES

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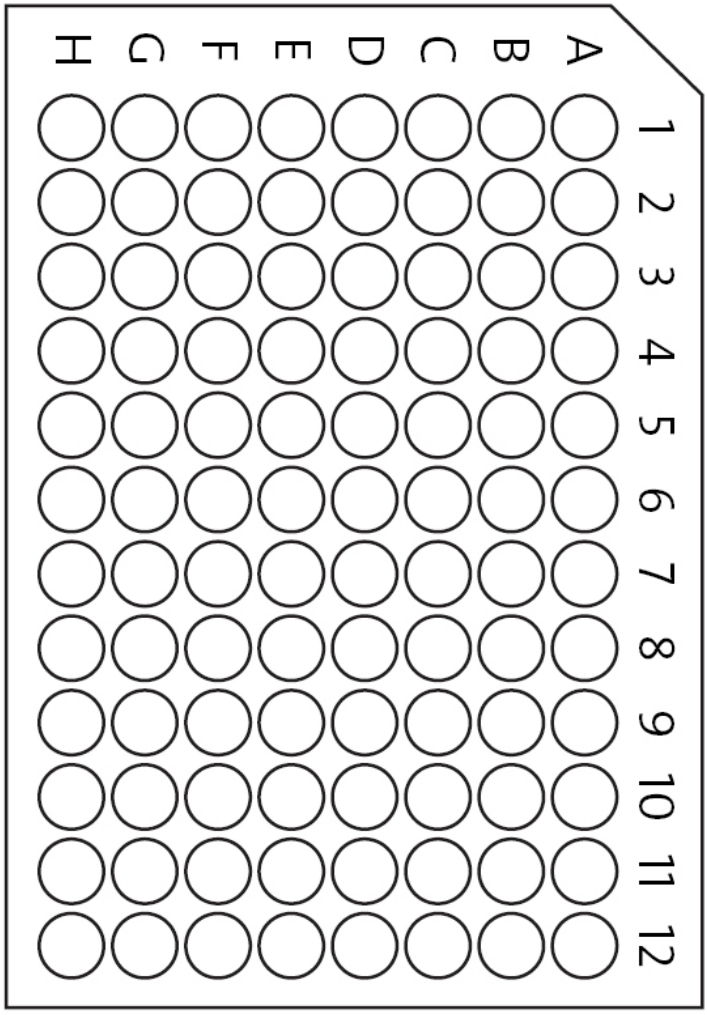
ASSAY DIAGRAM



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