

Instruction Manual No. M-400

**Beta Human Chorionic Gonadotropin
(HCG)**

**For Quantitative Determination of
Beta HCG In Human Serum**

For In Vitro Research Use Only

Beta-HCG ELISA KIT Cat. No. 0400 (96 Tests)

Kit Components	96 Tests
Anti-Beta HCG Coated Strip plates (96 wells).	Cat.# 401
Std. A , (sample diluent or Zero Std), 11 ml	Cat. # 402
Std. B , 5 mIU/ml, 0.75 ml	Cat. # 403
Std. C , 25 mIU/ml, 0.75 ml	Cat. # 404
Std. D , 50 mIU/ml, 0.75 ml	Cat. # 405
Std. E , 100 mIU/ml, 0.75 ml	Cat. # 406
Std. F , 200 mIU/ml, 0.75 ml	Cat. # 407
Standards are calibrated against WHO 3rd 75/537	
Anti-HCG-HRP Conjugate , 11 ml	Cat # 4 0 8
HRP Substrate Solution A , 11 ml	4 0 0 S A
HRP Substrate Solution B , 11 ml	4 0 0 S B
Wash Buffer (20X), 50 ml	W B - 2 0
Stop solution, 10 ml	T - 1 0
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Introduction

HCG molecule consists of two non-covalently linked polypeptide subunits, the alpha and beta subunits. The alpha subunit is common with many other peptide hormones such as TSH, LH, and FSH. The differences in the amino acid sequences of the beta subunits of a given hormone determine its biological functions. Therefore, specific antibodies made to the beta subunits are used for the specific determination of HCG and other hormones.

Human Chorionic Gonadotropin is a glycoprotein hormone produced by the trophoblastic cells of the developing placenta at the time of blastocyst implantation. The appearance of HCG in serum and urine, as early as 7-10 days after conception and its rapid rise in concentration are the most reliable indication for early diagnosis of pregnancy. In normal non-pregnant women and men, serum HCG levels are usually below <5 IU/L. In normal second trimester maternal sera, the level of intact HCG ranges from 20,000-50,000 IU/ml. HCG measurements can also be used to monitor abnormal pregnancies, including ectopic pregnancies. In an ectopic pregnancy, HCG levels increase at lower rates and remain at lower levels than in normal pregnancy. Elevated levels of HCG are also seen in patients with neoplasm such as hydatiform mole and choriocarcinoma, Ovarian carcinoma and testicular carcinoma may also produce higher amounts of HCG. Determination of serum HCG can also be used as prenatal screening test for trisomy 21 (down's syndrome), often in combination with determination of AFP and free estriol. In the case of Down's syndrome pregnancies, high HCG levels are also found.

PERFORMANCE CHARACTERISTICS

1. DETECTION LIMIT

Based on sixteen replicate determinations of the zero standard, the minimum concentration of human HCG detected using this assay is 1.5 mIU/ml. The detection limit is defined as the value deviating by 2 SD from the zero standard.

2. PRECISION

Intra-assay precision:

Four serum samples (HCG concentrations 15.4, 44.8, 119.9 mIU/ml) were run in 10 assays. The samples showed good intra-assay precision with % CV of 10.8, 6.4, and 6.6, respectively.

Inter-assay precision:

Three serum samples were run in duplicate in sixteen independent assays. The samples showed good inter-assay precision (5-8.2 % CV). The actual values were: mean 12.0 mIU/ml, SD 0.84 mIU/ml, % CV 6.9; mean 29.4 mIU/ml, SD 1.5 mIU/ml, % CV 5.0; mean 86.3 mIU/ml, SD 7.0 mIU/ml, % CV 8.2, and mean 127 mIU/ml, SD 6.7 mIU/ml, % CV 5.2, respectively.

3. RECOVERY

A known amount of HCG (10, 50, and 100 mIU/ml) was added to three patient sera (with original HCG concentrations of 12.7, 42, and 114 mIU/ml) and the total HCG levels measured. The assay showed excellent mean recoveries of about 102% (range 93-106%).

4. LINEARITY

Four different patient samples (with original HCG concentrations of 13, 45, 136, and 220 mIU/ml) were diluted (1:2, 1:5, and 1:10) with the zero standard and their final HCG values determined. The samples showed excellent mean recoveries of about 102% (range 96-105%).

5. SPECIFICITY

The specificity of HCG ELISA kit was determined by measuring interference from high concentrations of various related hormones: hLH (up to 200 mIU/ml produced color equivalent to <2 HCG mIU/ml), hFSH (up to 300 mIU/ml; produced color equivalent to <3 HCG mIU/ml), and hTSH (up to 50 uIU/ml; produced color equivalent to <6 HCG mIU/ml). These hormones had a minimal interference in the HCG assay

6. HIGH DOSE HOOK EFFECT

HCG concn. of up to 40000 mIU/ml did not cause any hook effect in this assay.

STORAGE AND STABILITY

The microtiter well plate and all other reagents are stable at 2-8°C until the expiration date printed on the label. The whole kit stability is usually 6 months from the date of shipping under appropriate storage conditions. The unused portions of the standards should be stored at 2-8°C.

TEST PROCEDURE (ALLOW ALL REAGENTS TO REACH ROOM TEMPERATURE BEFORE USE).

Remove required number of coated strips and arrange them on the plate. Store unused strips in the bag. Dispense 200-300 µl of wash buffer to all wells. Mix for **5 seconds** and discard or aspirate the solution. The step should be done just before adding the samples, do not allow the wells to dry at any time during the assay. **Dilute wash buffer 1:20** with water (50 ml concn in 950 ml water).

1. Label or mark the microtiter well strips to be used on the plate.
2. Pipet **25 µl** of standards, control, and serum samples into appropriate wells in *duplicate*.
3. Dispense **100 µl** of enzyme-conjugate into each well.
4. Mix gently for 5-10 seconds, cover the plate and incubate at room temperature for **30 minutes**.
5. Aspirate and wash the wells **5 times** with 300 µl of wash buffer. We recommend using an automated ELISA plate washer for better consistency. Failure to wash the wells properly will lead to high blank or zero values. If washing manually, plate must be tapped over paper towel between washings to ensure proper washing.
6. Premix required volume of TMB substrate Solns. A and B (1:1, v/v) in a separate clean tube and dispense and 200 µl added into each well. Mix gently for 5-10 seconds, cover the plate and incubate at room temp. for **10 minutes**. It is possible to increase or decrease the incubation time by 5 minutes to achieve the maximum A450 of ~2.0.
7. Stop the reaction by adding 50 µl of stopping solution to **all wells**. Mix gently for 5-10 seconds and read the plate at 450nm within 15-30 min.

NOTES

Read instructions carefully before the assay. Do not allow reagents to dry on the wells. Careful aspiration of the washing solution is essential for good assay precision. Since timing of the incubation steps is important to the performance of the assay, pipet the samples without interruption and it should not exceed 5 minutes to avoid assay drift. If more than one plate is being used in one run, it is recommended to include a standard curve on each plate. The unused strips should be stored in a sealed bag at 4°C. Addition of the HRP substrate solution starts a kinetic reaction, which is terminated by dispensing the stopping solution. Therefore, keep the incubation time for each well the same by adding the reagents in identical sequence. Plate readers measure absorbance vertically. Do not touch the bottom of the wells.

DILUTION OF SAMPLES

Serum samples containing more than 200 mIU/ml HCG must be diluted with the zero standard (standard A or normal saline) and the results obtained should be multiplied by the appropriate dilution factor.

CALCULATION OF RESULTS

Calculate the mean absorbance for each duplicate. Subtract the absorbance of the zero standard from the mean absorbance values of standards, control, and samples. Draw the standard curve on log-log graph paper by plotting net absorbance values of standards against appropriate HCG concentrations. Read off the HCG concentrations of the control and patient samples.

Expected Values

Serum HCG levels during pregnancy are estimated to be:

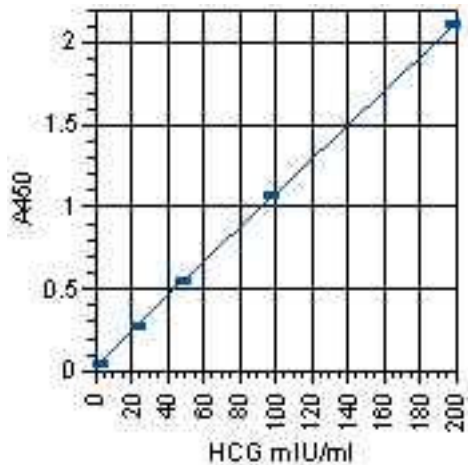
Time of post conception	mIU/ml
1 week	5-50
2 weeks	40-200
3 weeks	100-500
4 weeks	700-1000
2-3 months	12000-200000
2 nd trimester	24000-55000
3 rd Trimester	6000-48000

During the first 6 weeks of pregnancy, serum hCG conc have a doubling time of ~2 days. Following delivery, HCG conc rapidly decrease and usually return to normal level in several days. High levels of HCG may persist after HCG injection in patients undergoing infertility therapy. HCG may be present in very low levels in ectopic pregnancies while conditions like choriocarcinoma, trophoblastic or nontrophoblastic neoplasm, or hydatiform mole may result in high concn of HCG.

WORKSHEET OF TYPICAL ASSAY

Wells	Stds/samples	Mean A_{450nm}	Calculated Conc. (mIU/ml)
A1, A2	Std. A (0 mIU/ml)	0.004	
B1, B2	Std. B (5 mIU/ml)	0.052	
C1, C2	Std. C (25 mIU/ml)	0.285	
D1, D2	Std. D (50 mIU/ml)	0.575	
E1, E2	Std. E (100 mIU/ml)	1.095	
F1, F2	Std. F (200 mIU/ml)	2.100	
G1, G2	Sample 1	1.533	140

NOTE: These data are for demonstration purpose only. A complete standard curve must be run in every assay to determine sample values. Each laboratory should determine their own normal reference values.



A typical std. assay curve (do not use this for calculating sample values)

PRINCIPLE OF THE TEST

β -HCG ELISA kit is based on sequential binding of β -HCG from patient samples to two antibodies, one immobilized on microtiter well plates, and other (specific for the α -chain of HCG) conjugated to the enzyme horseradish peroxidase. After a washing step, chromogenic substrate is added and color (blue converted to yellow) developed. The enzymatic reaction (yellow color) is directly proportional to the amount of β -HCG present in the sample. Adding stopping solution terminates the reaction. Absorbance is then measured on a microtiter well ELISA reader at 450 nm. The unknown sample values are then read-off the standard curve.

MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipet (20-100 μ l) and Multichannel pipet with disposable plastic tips. Reagent troughs, Plate washer (recommended) and ELISA plate Reader.

PRECAUTIONS

The HCG ELISA kit is intended for *in vitro* research use only. The reagents contain proclin-300 (0.1) as preservative; necessary care should be taken when disposing solutions. The Controls and Standards have been prepared from human sera shown to be negative for HBsAg and HIV antibodies. Nevertheless, such tests are unable to prove the complete absence of viruses, therefore, sera should be handled with appropriate precautions.

Applicable MSDS, if not already on file, for the following reagents can be obtained from ADI or the web site.

TMB (substrate), H₂SO₄ (stop solution), and Proclin-300 (0.1% v/v in standards, sample diluent and HRP-conjugates).

All waste material should be properly disinfected before disposal. Avoid contact with the stop solution (1N sulfuric acid).

SPECIMEN COLLECTION AND HANDLING

Collect blood by venipuncture, allow to clot, and separate the serum by centrifugation at room temperature. Do not heat inactivate the serum. If sera can not be immediately assayed, these could be stored at -20°C for up to six months. Avoid repeated freezing and thawing of samples. No preservatives should be added to the serum.

Reagent Preparation

Dilute wash buffer concentrate 1:20 (50 ml stock and 950 ml water) with distilled water. Keep working solution at room temp for 1-2 days and at 4°C for long term storage.