hCG ELISA Kit

For the quantitative determination of intact human chorionic gonadotropin (hCG) concentrations in serum

Catalog Number: AAA14617

96 tests

FOR LABORATORY RESEARCH USE ONLY
NOT FOR DIAGNOSTIC USE

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INTENDED USE

This HCG ELISA Kit is to be used for the *in vitro* quantitative determination of intact human chorionic gonadotropin (hCG) concentrations in serum. This kit is intended FOR LABORATORY RESEARCH USE ONLY and is not to be used for diagnostic or therapeutic procedures.

INTRODUCTION

Human chorionic gonadotropin is a glycoprotein hormone produced by normal trophoblast cells of the placenta during pregnancy. It is also is produced by trophoblast cells in hydatidiform mole and choriocarcinoma (trophoblast diseases), and in patients with germ cell tumors (testicular choriocarcinoma, placental site tumors and germ cell carcinomas of the ovary) and sometimes in those with other malignancies. Small amount of hCG may also be produced by the pituitary gland. Human CG (hCG) is the hormone associated with the maintenance of pregnancy.

Human chorionic gonadotropin is a member of the glycoprotein hormone (GPH) family. Like the other glycoprotein hormones, follicle stimulating hormone (FSH), luteinizing hormone (LH) and thyroid stimulating hormone (TSH), hCG is composed of two subunits, alpha and beta. Alpha subunits of these various glycoprotein hormones are structurally very similar, but beta subunits differ in amino acid sequences. These differences are responsible for their biological and immunological specificity. The alpha-subunit of hCG is a glycopeptide of 92 amino acids and the ß-subunit of hCG is a glycopeptide of 145 amino acids. HCG has a molecular weight of 50,000 daltons, consists of an alpha subunit of 18,000 daltons and a beta subunit of 32,000 daltons. Recent elucidation of the crystal structure of hCG has revealed that all these subunits share the so-called cystin-knot structural motif with growth factors such as nerve (NGF), platelet-derived (PDGF). Although the pituitary secretes three related glycoprotein hormones, LH, FSH, and TSH, hCG is the only one to produced by the placenta in primates to maintain the steroid hormone secretions of the corpus luteum.

Predominantly intact hCG is present in serum and urine samples in normal pregnancy, as well as in hydatidiform mole. In women with extrauterine or ectopic pregnancies, unduly low hCG levels may be detected. Variable levels of hCG mostly intact hCG, may be present from individuals with persistent trophoblastic disease. Elevated concentrations are also present in cases of early pregnancy loss (EPL) or biochemical pregnancy, gestational Down syndrome. Bladder cancer, ovarian cancer and certain other malignancies may generate a small amount of hCG alpha and beta subunit. Commonly, the amount of subunit is insufficient for combination to occur to make intact hCG.

PRINCIPLES OF THE ASSAY

This hCG enzyme linked immunosorbent assay (ELISA) applies a technique called a quantitative sandwich immunoassay. The microtiter plate provided in this kit has been pre-coated with a monoclonal antibody specific to hCG. Standards or samples are added to the microtiter plate wells and incubated. The plate wells are washed to remove any unbound standard or sample, hCG, if present, will bind to the antibody pre-coated on the wells. A standardized preparation of horseradish peroxidase (HRP)-conjugated monoclonal antibody specific to hCG is added to each well to “sandwich” the hCG immobilized on the plate. The microtiter plate undergoes incubation, and then the wells are thoroughly washed to remove all unbound components. Next, a TMB (3,3', 5,5' Tetramethyl-benzidene) substrate solution is added to each well. This enzyme (HRP) and substrate are allowed to react over a short incubation period. Only those wells that contain hCG and enzyme-conjugated antibody will exhibit a change in colour. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the colour change is measured spectrophotometrically at a wavelength of 450 nm.

In order to measure the concentration of hCG in the sample, this ELISA Kit includes a set of calibration standards (6 standards). The calibration standards are assayed at the same time as the samples and allow the operator to produce a standard curve of Optical Density (O.D.) versus hCG concentration (mIU/mL). The concentration of hCG in the samples is then determined by comparing the O.D. of the samples to the standard curve.

REAGENTS PROVIDED

All reagents provided are stored at 2-8°C. Refer to expiration date on the label.

	96 tests

1. **HCG MICROTITER PLATE** (Part EL31-1) ***96 wells***
   Pre-coated with anti-human hCG monoclonal antibody.
2. **CONJUGATE** (Part EL31-2) ***12 mL***
   Anti-hCG monoclonal antibody conjugated to horseradish peroxidase (HRP) with preservative. *Ready to use.*
3. **HCG STANDARD - 240 mIU/mL** (Part EL31-3) ***1 vial***
   Lypholized human hCG in a buffered protein base with preservative that will contain 240 mIU/mL after reconstitution.
4. **HCG STANDARD - 120 mIU/mL** (Part EL31-4) ***1 vial***
   Lypholized human hCG in a buffered protein base with preservative that will contain 120 mIU/mL after reconstitution.
5. **HCG STANDARD - 60 mIU/mL** (Part EL31-5) ***1 vial***
   Lypholized human hCG in a buffered protein base with preservative that will contain 60mIU/mL after reconstitution.
6. **HCG STANDARD - 30 mIU/mL** (Part EL31-6) ***1 vial***
   Lypholized human hCG in a buffered protein base with preservative that will contain 30mIU/mL after reconstitution.
7. **HCG STANDARD - 7.5 mIU/mL** (Part EL31-7) ***1 vial***
   Lypholized human hCG in a buffered protein base with preservative that will contain 7.5 mIU/mL after reconstitution
8. **HCG STANDARD - 0 mIU/mL** (Part EL31-8) ***1 vial***
   Lypholized human hCG in a buffered protein base with preservative that will contain 0 mIU/mL after reconstitution.
9. **SUBSTRATE A** (Part EL31-9) ***10 mL***
   Buffered solution with H_2O_2.
10. **SUBSTRATE B** (Part 30007) ***10 mL***
    Buffered solution with TMB.
11. **STOP SOLUTION** (Part 30008) ***14 mL***
    2N H_2SO_4. Caution: Caustic Material!
12. **SAMPLE DILUENT**(Part EL31-10) ***10 mL***

MATERIALS REQUIRED BUT NOT SUPPLIED

1. Single or multi-channel precision pipettes with disposable tips: 10-100µL and 50-200µL for running the assay.
2. Pipettes: 1 mL, 5 mL 10 mL, and 25 mL for reagent preparation.
3. Multi-channel pipette reservoir or equivalent reagent container.
4. Test tubes and racks.
5. Polypropylene tubes or containers (25 mL).
6. Incubator (37°C).
7. Microtiter plate reader (450 nm ± 2nm)
8. Automatic microtiter plate washer or squirt bottle.
9. Sodium hypochlorite solution, 5.25% (household liquid bleach).
10. Deionized or distilled water.
11. Plastic plate cover.
12. Disposable gloves.
13. Absorbent paper.

PRECAUTIONS

1. Do not substitute reagents from one kit lot to another. Standards, conjugate and microtiter plates are matched for optimal performance. Use only the reagents supplied by manufacturer.
2. Allow kit reagents and materials to reach room temperature (20-25°C) before use. Do not use water baths to thaw samples or reagents.
3. Do not use kit components beyond their expiration date.
4. Use only deionized or distilled water to dilute reagents.
5. Do not remove microtiter plate from the storage bag until needed. Unused strips should be stored at 2-8°C in their pouch with the desiccant provided.
6. Use fresh disposable pipette tips for each transfer to avoid contamination.
7. Human serum and plasma should be handled as potentially hazardous and capable of transmitting disease. Disposable gloves must be worn during the assay procedure since no known test method can offer complete assurance that products derived from human blood will not transmit infectious agents. Therefore, all blood derivatives should be considered potentially infectious and good laboratory practices should be followed.
8. All samples should be disposed of in a manner that will inactivate human viruses.
   Solid Waste: Autoclave 60 min. at 121°C.
   Liquid Waste: Add sodium hypochlorite to a final concentration of 1.0%. The waste should be allowed to stand for a minimum of 30 minutes to inactivate viruses before disposal.
9. Substrate Solution is easily contaminated. If bluish prior to use, *do not use*.
10. The Substrate B contains 20% acetone, keep reagent away from sources of heat or flame.

SAMPLE PREPARATION

COLLECTION, HANDLING AND STORAGE

Serum: Blood should be drawn using standard venipuncture techniques and serum separated from blood cells as soon as possible. Samples should be allowed to clot for one hour at room temperature, centrifuged for 10 minutes (4°C) and serum extracted. This kit is for use with serum samples without additives only.

- Avoid grossly hemolytic, lipidic or turbid samples.
- Serum samples to be used within 24-48 hours may be stored at 2-8°C otherwise samples must be stored at -20°C to avoid loss of bioactivity and contamination. Avoid freeze-thaw cycles.
- When performing the assay slowly bring samples to room temperature.
- It is recommended that all samples be assayed in duplicate.
- DO NOT USE HEAT-TREATED SPECIMENS.

PREPARATION OF REAGENTS

Remove all kit reagents from refrigerator and allow them to reach room temperature (20-25°C). Prepare the following reagents as indicated below. Mix thoroughly by gently swirling before pipetting. Avoid foaming.

1. **HCG Standards:** Reconstitute each HCG Standard vial with **0.6 mL** of deionized or distilled water. Allow each solution to sit for at least 15 minutes with gentle agitation. The HCG standard stock solutions are stable at 4°C for 3 months. Avoid freeze-thaw cycles.

2. **Substrate Solution:** Substrate A and Substrate B should be mixed together in equal volumes up to 15 minutes before use. Refer to the table below for correct amounts of Substrate Solution to prepare.

Wells Used	Substrate A (mL)	Substrate B (mL)	Substrate Solution (mL)
16 wells	1.5	1.5	3.0
32 wells	3.0	3.0	6.0
48 wells	4.0	4.0	8.0
64 wells	5.0	5.0	10.0
80 wells	6.0	6.0	12.0
96 wells	7.0	7.0	14.0

ASSAY PROCEDURE

1. Prepare all hCG Standards before starting assay procedure (see Preparation Reagents). *It is recommended that all Standards and Samples be added in duplicate to the Microtiter Plate.*

2. First, secure the desired number of coated wells in the holder, then add 50 μL of Standards or Samples to the appropriate well of the antibody pre-coated Microtiter Plate. Then add 50 μL of Sample Diluent to each well. COMPLETE MIXING IN THIS STEP IS IMPORTANT. Cover and incubate for **30 minutes at 37ºC**.

3. Wash the Microtiter Plate using one of the specified methods indicated below:

   Manual Washing: Remove incubation mixture by aspirating contents of the plate into a sink or proper waste container. Using a squirt bottle, fill each well completely with de-ionized or distilled water, and then aspirate contents of the plate into a sink or proper waste container. Repeat this procedure four more times for a **total of FIVE washes**. After final wash, invert plate, and blot dry by hitting plate onto absorbent paper or paper towels until no moisture appears. *Note*: Hold the sides of the plate frame firmly when washing the plate to assure that all strips remain securely in frame.

   Automated Washing: Aspirate all wells, then wash plates **FIVE times** using distilled or de-ionized water. Always adjust your washer to aspirate as much liquid as possible and set fill volume at 350 μL/well/wash (range: 350-400 μL). After final wash, invert plate, and blot dry by hitting plate onto absorbent paper or paper towels until no moisture appears. *It is recommended that the washer be set for soaking time of 10 seconds or shaking time of 5 seconds between washes.*

4. Add 100 μL of Conjugate into each well. Cover and incubate for **30 minutes at 37ºC.**

5. Repeat wash procedure as described in Step3.

6. Add 100μL of TMB Substrate into each well. Cover and incubate for **15 minutes at 37ºC**.

7. Add 100 μL of Stop Solution to each well. Mix well.

8. Read the Optical Density (O.D.) at 450 nm using a microtiter plate reader within 30 minutes.

CALCULATION OF RESULTS

This standard curve is used to determine the amount of hormone (hCG) in an unknown sample. The standard curve is generated by plotting the average O.D. (450 nm) obtained for each of the six standard concentrations on the vertical (Y) axis versus the corresponding hCG concentration (mIU/mL) on the horizontal (X) axis.

1. First, calculate the mean O.D. value for each standard and sample. All O.D. values are subtracted by the mean value of the zero-standard (0 mIU/mL) before result interpretation. Construct the standard curve using graph paper or statistical software.

2. To determine the amount of hCG in each sample, first locate the O.D. value on the Y-axis and extend a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the corresponding hCG concentration.

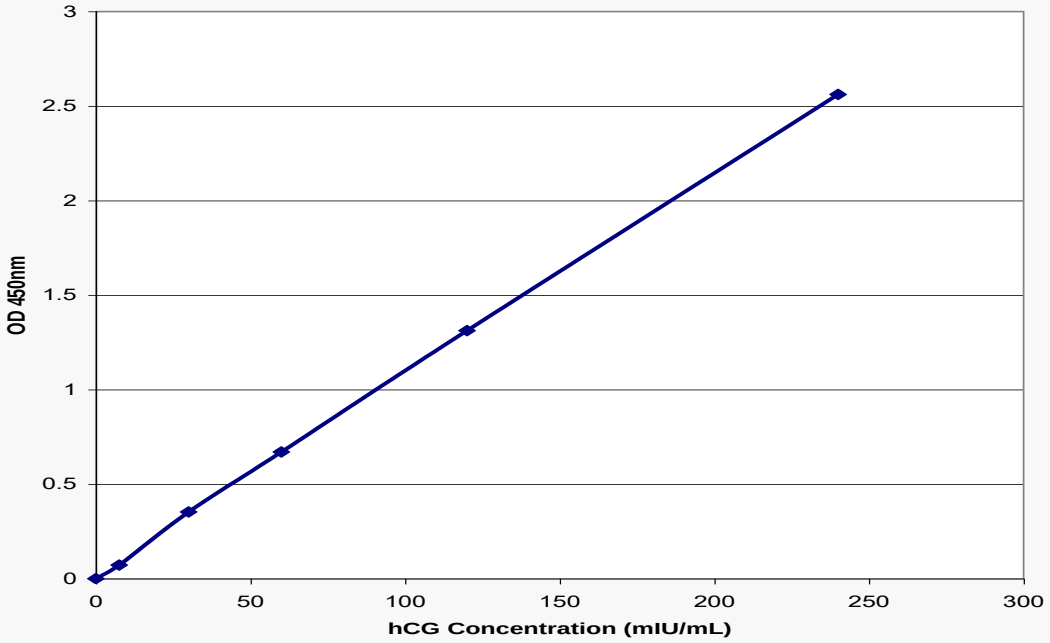
TYPICAL DATA

Results of a typical standard run of hCG ELISA are shown. Any variation in operator, pipetting and washing technique, incubation time or temperature, and kit age can cause variation in result. The following examples are for the purpose of illustration only, and should not be used to calculate unknowns. Each user should obtain their own standard curve.

EXAMPLE

Results of a typical standard run are shown below:

HCG (mIU/mL)	O.D. (450 nm)	Mean	Zero Standard Subtracted
0	0.048, 0.050	0.049	0
7.5	0.121, 0.120	0.121	0.072
30	0.400, 0.404	0.402	0.353
60	0.718, 0.720	0.719	0.670
120	1.361, 1.360	1.361	1.312
240	2.600, 2.620	2.610	2.561



PERFORMANCE CHARACTERISTICS

1. **SENSITIVITY**
   The minimal detectable concentration of HCG by this assay is estimated to be 3.0mIU/mL.

2. **SPECIFICITY**
   This kit exhibits no detectable cross reactivity with FSH, TSH, or Prolactin. This kit has slight cross reactivity with hLH (3%).

3. **CALIBRATION**
   This immunoassay is calibrated against NIBSC/WHO, 3rd International Standard Code 75/537.

4. **PRECISION**
   The Intra-assay variations were assessed by testing samples in 16 wells within one assay.

N=16	Mean value (mIU/mL)	Coefficient of variation
Sample 1	37.2	5.95
Sample 2	198.46	6.16

The Inter-assay variations were assessed by testing samples in 8 different assays with assay kits from three different lots.

N=8	Mean value (mIU/mL)	Coefficient of variation
Sample 1	47.25	7.59
Sample 2	217.25	12.60

5. **RECOVERY**
   The sample recovery rates were assessed with 2 blood samples spiked with 65IU/mL HCG.

	Dilution	Expected value (mIU/mL)	Measured value (mIU/mL)	Rate of recovery
Sample 1 (Serum)	780	83	87	105%
Sample 2 (Plasma)	780	83	74	88%

6. **HOOK EFFECT**
   In this assay, no hook effect is observed.

7. **EXPECTED NORMAL VALUES**
   Each laboratory must establish its own normal range 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 100,000-200,000mIU/mL at the end of the first trimester.

CITATIONS

1. van den Heuvel MJ, Hatta K, Peralta CG, Han VK, Clark DA. CD56+ cells are recruited to the uterus in two waves: at ovulation and during the first 2 weeks after missed menses. Am J Reprod Immunol. 2008 59(2):90-8

2. Ma QY et al., High levels of chorionic gonadotrophin attenuate insulin sensitivity and promote inflammation in adipocytes. J Mol Endocrinol 2015 2(54):161-170