ELISA kits available from ADI (see details at the web site)

#0010	Human Leptin		
#200-120-AGH	Human globular Adiponectin (gAcrp30)		
#0700	Human Sex Hormone Binding Glob (SHBG)		
#0900	Human IGF-Binding Protein 1 (IGFBP1)		
#1000	Human C-Reactive Protein (CRP)		
#100-110-RSH	Human Resistin /FIZZ3		
#100-140-ADH	Human Adiponectin (Acrp30)		
#100-160-ANH	Human Angiogenin		
#100-180-APH	Human Angiopoietin-2 (Ang-2)		
#100-190-B7H	Human Bone Morphogenic Protein 7 (BMP-7)		
#1190	Human Serum Albumin	**#1200**	Human Albumin (Urinary)
#1750	Human IgG (total)	**#1760**	Human IgM
#1800	Human IgE	**#1810**	Human Ferritin
#1210	Human Transferrin (Tf)	**#0020**	Beta-2 microglobulin
#1600	Human Growth Hormone (GH)		

#0060	Human Pancreatic Colorectal cancer (CA-242)		
#1820	Human Ovarian Cancer (CA125)	**#1830**	Human CA153
#1840	Human Pancreatic & GI Cancer (CA199)		
#1310	Human Pancreatic Lipase		
#1400	Human Prostatic Acid Phosphatase (PAP)		
#1500	Human Prostate Specific Antigen (PSA)	**#1510**	free PSA (fPSA)
#0500	Human Alpha Fetoprotein (AFP)		
#0050	Human Neuron Specific Enolase (NSE)		

#0030	Human Insulin	**#0040**	Human C-peptide
#0100	Human Luteinizing Hormone (LH)		
#0200	Human Follicle Stimulating Hormone (FSH)		
#0300	Human Prolactin (PRL)		
#0400	Human Chorionic Gonadotropin (HCG)	#**0410**	HCG-free beta

#0600	Human Thyroid Stimulating Hormone (TSH)		
#1100	Human Total Thyroxine (T4)	**#1110**	Human Free T4 (fT4)
#1650	Human free triiodothyronine (fT3)	**#1700**	Human T3 (total)

#1850	Human Cortisol	**#1860**	Human Progesterone
#1865	Human Pregnolone	**#1875**	Human Aldosterone
#1880	Human Testosterone	**#1885**	Human free Testosterone
#1910	Human Androstenedione	**#1920**	Human Estradiol
#1925	Human Estrone	**#1940**	Dihydrotestosterone (DHT)
#1950	Human DHEA-sulphate (DHEA-S)		
#3400	Human serum Neopterin		

#3000	Human Rheumatoid Factors IgM (RF)
#3100	Human anti-dsDNA
#3200	Anti-Nuclear Antibodies (ANA) \

Instruction Manual No. M-400

Human Chorionic Gonadotropin (HCG)

ELISA KIT Cat. No. 0400, 96 Tests

For Quantitative Determination of HCG In Human Serum

For In Vitro Research Use Only

ALPHA DIAGNOSTIC INTERNATIONAL

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

Kit Components	96 Tests
Anti- HCG Coated Strip plates (96 wells).# 401	1 Plate
Standard A, (0 IU/L) 2 ml #402A	1 vial
Standard B,(2.5 IU/L), 0.5 ml, # 402B	1 vial
Standard C, (10 IU/L), 0.5 ml, # 402C	1 vial
Standard D, (25 IU/L), 0.5 ml, # 402D	1 vial
Standard E, (100 IU/L), 0.5 ml, # 402E	1 vial
Standard F, (500 IU/L), 0.5 ml, # 402F	1 vial
HCG **Low & High controls**, 0.5 ml/vial, exact values printed on vial, #LC-400, #HC-400	
Anti-HCG-HRP **Conjugate (50X)**, 0.3 ml , #403; dilute 1:50 in assay buffer	1 vial
Assay Buffer, 25 ml, #404	1 bottle
HRP Substrate **Soln**, (Ready-to-use) 16 ml, TMB-400	1 bottle
Wash Buffer (10X), 50 ml **(dilute 1:10 with distilled water), WB-10**	1 bottle
Stop solution, 6 ml, ST-400	1 bottle
Complete Instruction Manual	1

Intended Use: ADI's HCG ELISA kit is for quantitative determination of HCG In human Serum. ***For In Vitro Research Use Only (RUO)***

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 form 20,0000-50,000 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 0.7 IU/ml or 0.7mIU/ml. The detection limit is defined as the value deviating by 2 SD from the zero standard.

2. PRECISION

Intra-assay precision:

Sample	Mean (IU/L)	**SD**	**CV%**
1	4.52	0.24	5.3
2	18.80	0.60	3.2
3	121.84	5.36	4.4

Inter-assay precision:

Sample	Mean (IU/L)	**SD**	**CV%**
1	5.24	0.36	6.9
2	14.68	0.72	4.9
3	140.27	11.50	8.2

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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 50-200 IU/L produced color equivalent to <5 HCG IU/L), hFSH (up to 4000 IU/L; produced color equivalent to <5 HCG IU/L), and hTSH (up to 50-750 IU/L; produced color equivalent to <5 HCG IU/L). 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^{o}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^{o}C.

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

Dilute wash buffer (1:10) with distilled water (50 ml stock in 450 ml). Prepare 1X HRP conjugate solution (dilute 1:50 with assay buffer)

Remove required number of coated strips and arrange them on the plate. Store unused strips in the bag.

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 ul** of diluted enzyme-conjugate into each well. Mix gently for 5-10 seconds, cover the plate and incubate on a plate shaker (approx 200 rpm) at room temperature (25-28oC) for **60 minutes.**

4. Aspirate and wash the wells **3 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.

5. Dispense **100 ul TMB substrate per well.** Mix gently. cover the plate and incubate on a plate shaker (approx 200 rpm) at room temperature for **15 minutes.** Blue color develops in standard/controls and positive wells. **Note:** The incubation time can be varied ±5 min to get the maximum A450 of the highest standard at 2.5-3.0 or within the reading range of the ELISA reader.

6. Stop the reaction by adding **50 µl** of stopping solution to **all wells**. Mix gently for 5-10 seconds. Bluer color turns yellow. 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^{o}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 500 IU/L or 500 mIU/ml HCG must be diluted with the zero standard (standard A or assay buffer) and the results obtained should be multiplied by the appropriate dilution factor.

Expected Values

As for all clinical assays each laboratory must determine its own reference values. The following data should only be used as guidelines.

Samples	IU/L
Males	<5
Females (non-pregnant)	<5
Females (pregnant)	>250

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.

List of Publication using ADI HCG ELISA Kits

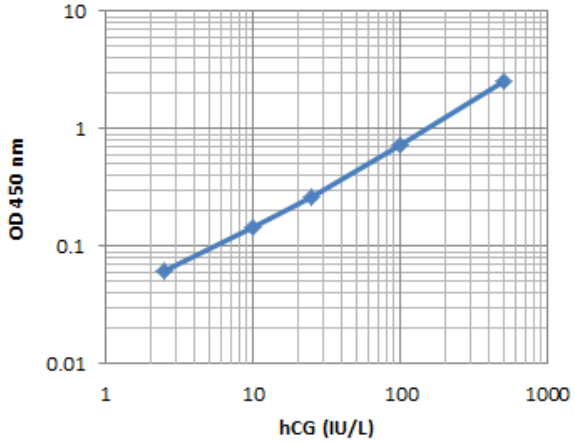
Ivnitski D 2004 Analytica Chimica Acta 504, 265-269
HCG/sensor assay- saliva
Chan D 2004 J. Pharmacol. Exp. Ther., 310(1):75-82
hcg in tissue lysate

Jackson SW 2007 Am. J. Pathol., Oct 2007; 171: 1395 – 1404
mice inoculated with B16-CG melanoma cells measured beta-HCG in mouse urine

WORKSHEET OF TYPICAL ASSAY

Wells	Stds/samples	Mean A_{450nm}
A1, A2	**Std. A** (0 IU/L)	0.074
B1, B2	**Std. B** (2.50 IU/L)	0.092
C1, C2	**Std. C** (10 IU/L)	0.145
D1, D2	**Std. D** (25 IU/L)	0.259
E1, E2	**Std. E** (100 IU/L)	0.719
F1, F2	**Std. F** (500 IU/L)	2.499

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.



Kit-spec-XL

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

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. All samples reading at or above highest standards must be diluted and restested. If ELISA reader software is being used, we recommend 4-paramter or 5-parameter curve.

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 µl) and Multichannel pipet with disposable plastic tips. Reagent troughs, Plate washer (recommended) and ELISA plate Reader.

PRECAUTIONS

The Alpha Diagnostic International β-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^{o}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 (1:10) with distilled water (50 ml stock in 450 ml. Store at 4oC.

Prepare 1X solution HRP conjugate. **Dilute 20 ul stock conjugate per ml of assay buffer**. (**200 ul in 10 ml** for complete 96-well plate).