

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

<b>#0010</b>	Human Leptin		
<b>#200-120-AGH</b>	Human globular Adiponectin (gAcrp30)		
<b>#0700</b>	Human Sex Hormone Binding Glob (SHBG)		
<b>#0900</b>	Human IGF-Binding Protein 1 (IGFBP1)		
<b>#1000</b>	Human C-Reactive Protein (CRP)		
<b>#100-110-RSH</b>	Human Resistin /FIZZ3		
<b>#100-140-ADH</b>	Human Adiponectin (Acrp30)		
<b>#100-160-ANH</b>	Human Angiogenin		
<b>#100-180-APH</b>	Human Angiopoietin-2 (Ang-2)		
<b>#100-190-B7H</b>	Human Bone Morphogenic Protein 7 (BMP-7)		
<b>#1190</b>	Human Serum Albumin	<b>#1200</b>	Human Albumin (Urinary)
<b>#1750</b>	Human IgG (total)	<b>#1760</b>	Human IgM
<b>#1800</b>	Human IgE	<b>#1810</b>	Human Ferritin
<b>#1210</b>	Human Transferrin (Tf)	<b>#0020</b>	Beta-2 microglobulin
<b>#1600</b>	Human Growth Hormone (GH)		
<b>#0060</b>	Human Pancreatic Colorectal cancer (CA-242)		
<b>#1820</b>	Human Ovarian Cancer (CA125)	<b>#1830</b>	Human CA153
<b>#1840</b>	Human Pancreatic & GI Cancer (CA199)		
<b>#1310</b>	Human Pancreatic Lipase		
<b>#1400</b>	Human Prostatic Acid Phosphatase (PAP)		
<b>#1500</b>	Human Prostate Specific Antigen (PSA)	<b>#1510</b>	free PSA (fPSA)
<b>#0500</b>	Human Alpha Fetoprotein (AFP)		
<b>#0050</b>	Human Neuron Specific Enolase (NSE)		
<b>#0030</b>	Human Insulin	<b>#0040</b>	Human C-peptide
<b>#0100</b>	Human Luteinizing Hormone (LH)		
<b>#0200</b>	Human Folicle Stimulating Hormone (FSH)		
<b>#0300</b>	Human Prolactin (PRL)		
<b>#0400</b>	Human Chorionic Gonadotropin (HCG)	<b>#0410</b>	HCG-free beta
<b>#0600</b>	Human Thyroid Stimulating Hormone (TSH)		
<b>#1100</b>	Human Total Thyroxine (T4)	<b>#1110</b>	Human Free T4 (ft4)
<b>#1650</b>	Human free triiodothyronine (ft3)	<b>#1700</b>	Human T3 (toal)
<b>#1850</b>	Human Cortisol	<b>#1860</b>	Human Progesterone
<b>#1865</b>	Human Pregnlone	<b>#1875</b>	Human Aldosterone
<b>#1880</b>	Human Testosterone	<b>#1885</b>	Human free Testosterone
<b>#1910</b>	Human Androstenedione	<b>#1920</b>	Human Estradiol
<b>#1925</b>	Human Estrone	<b>#1940</b>	Dihydrotestosterone (DHT)
<b>#1950</b>	Human DHEA-sulphate (DHEA-S)		
<b>#3400</b>	Human serum Neopterin		
<b>#3000</b>	Human Rheumatoid Factors IgM (RF)		
<b>#3100</b>	Human anti-dsDNA		
<b>#3200</b>	Anti-Nuclear Antibodies (ANA)		

Instruction Manual No. M-410

**Free Beta Human Chorionic Gonadotropin  
(Free  $\beta$  HCG)**

**ELISA KIT # 0410**

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

*For In Vitro Research Use Only*



**ALPHA DIAGNOSTIC  
INTERNATIONAL**

**India Contact:**

**Life Technologies (India) Pvt. Ltd.**  
306, Aggarwal City Mall, Opposite M2K Pitampura, Delhi – 110034  
Ph: +91-11-42208000, 42208111, 42208222

Mobile: +91-9810521400  
Fax: +91-11-42208444  
Email: [customerservice@atzlabs.com](mailto:customerservice@atzlabs.com)  
Web: [www.atzlabs.com](http://www.atzlabs.com)



**Free Beta Human Chorionic Gonadotropin (Free-Beta HCG)  
ELISA KIT Cat. No. 0410 (96 Tests)**

<b>Kit Components</b>	<b>96 Tests</b>
Anti-Beta HCG Coated Strip plates (96 wells). Cat.# 411	1 Plate
Free beta <b>Sample Diluent</b> , 13 ml, # 412	1 bottle
<b>Std. A</b> , 0 ng/ml, Lyophilized #413A	1 vial
<b>Std. B</b> , 2.5 ng/ml, Lyophilized #413B	1 vial
<b>Std. C</b> , 5 ng/ml, Lyophilized #413C	1 vial
<b>Std. D</b> , 10 ng/ml, Lyophilized #413D	1 vial
<b>Std. E</b> , 25 ng/ml, Lyophilized #413E	1 vial
<b>Std. F</b> , 50 ng/ml, Lyophilized #413F	1 vial
<b>Reconstitute each Lyophilized Standard with 1 ml distilled water.</b>	
Anti-free-beta HCG-HRP <b>Conjugate</b> , Cat # 414, 18 ml ready-to-use	1 bottle
TMB Substrate <b>Solution</b> , 11 ml #TMB-410	1 bottle
Wash buffer 100X, 10 ml #WB-410	1 bottle
Stop solution, Cat. # ST - 410, 11 ml	1 bottle
Complete Instruction Manual, M410	1

**Introduction**

Free beta HCG is a hormone produced by the placenta during pregnancy. The hormone is present in blood and urine around seven to 13 days following implantation of the fertilized ovum. Structurally intact HCG molecule consists of two non-covalently linked polypeptide subunits, the alpha and beta chain subunits. Measurements of intact HCG and of the alpha subunit of HCG appear to give similar results in blood and urine but not the levels of beta subunit.

In normal second trimester maternal sera, the level of intact HCG ranges from 20,000-50,000 mIU/ml. In contrast, the level of either free alpha or free beta-HCG is on average one half of 1% of HCG levels. HCG and the free subunits appear not to be useful as serological markers for nontrophoblastic tumors; however, the absolute increase of Beta-HCG level in choriocarcinoma patients clearly differentiates it from normal pregnancy.

Recent studies showed a significant increase in the level of free HCG-beta in trisomy 21 cases as compared with controls. Hence, it has been suggested that free Beta-HCG subunit assay in combination of maternal serum AFP could be effective in a screening for trisomy 21.

**PERFORMANCE CHARACTERISTICS**

**1. DETECTION LIMIT**

Based on sixteen replicates determinations of the zero standard, the minimum concentration of human free  $\beta$ -HCG detected using this assay is 0.5 mIU/ml. The detection limit is defined as the value deviating by  $2\pm$  SD from the zero standard.

**2. PRECISION**

*Intra-assay precision:*

Three serum samples (free  $\beta$ -HCG concentrations 16.5, 34, 90.1 mIU/ml) were run in 10 replicates. The samples showed good intra-assay precision with % CV of 10.3, 11.5, and 8.8, respectively.

*Inter-assay precision:*

Three serum samples were run in duplicate in 10 independent assays. The samples showed good inter-assay precision (9.2-10.3% CV). The actual values were: mean 17.4, 35.8, and 96.6 mIU/ml).

**3. SPECIFICITY**

The specificity of free  $\beta$ -HCG ELISA kit was determined by measuring interference from high concentrations of the following:

<b>Hormone tested</b>	<b>Conc. (mIU/ml)</b>	<b>Color intensity produced Equivalent of free <math>\beta</math>-HCG (mIU/ml)</b>
HCG	10,000	2
	1000	<2
	100	0
Alpha-HCG	100	0
hLH	200	<2
	80	0
TSH	75	0
FSH	200	0

**General References:** Birken S. Canfield RE Chemistry & Immunochemistry of HCG. In; Segal SJ Ed. Chorionic Gonadotropin. NY ; plenum Press: 65-88, 1980; Cole LA., Clinical Chem. 43:12 2233-2243, 1997, Ozturk M, Endocrinology. 120: 549-558, 1987; Macri JN, Am. J. Obstet. Gynecol. 163: 1248-1253, 1990.

**List of Publications using ADI HCG/Free-HCG ELISA kits**

Ivnitski D	2004	Analytica Chimica Acta 504, 265-269
Chan D	2004	J. Pharmacol. Exp. Ther., 310(1):75-82
Jackson SW	2007	Am. J. Pathol., Oct 2007; 171: 1395 - 1404
Chen Y.X.	2011	Int. J. Biochem. Ce.; Biol. 43, 821-828

## 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.

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

### Reconstitute each Lyophilized Standard with 1 ml distilled water.

1. Label or mark the microtiter well strips to be used on the plate.
2. Pipet **50 ul** of standards, control, and serum samples into appropriate wells in *duplicate*. Dispense **100 ul of zero buffer** into all wells. Mix gently for 5-10 seconds and **incubate at 37°C for 30 min**.
3. Wash 5-times with 1X wash buffer.
4. Dispense **150 ul enzyme conjugate** into each well. Mix gently for 5-10 seconds. Cover the plate and **incubate at 37°C for 30 minutes**.
5. Aspirate and wash the wells **5 times** with wash buffer, plate must be tapped over paper towel between washings to ensure proper washing.
6. Dispense **100 ul TMB substrate per well**. Mix the plate gently for 5-10 seconds. Cover the plate and **incubate at room temp. for 20 minutes**. Blue color develops in standards and positive wells.
7. Stop the reaction by adding **100 ul of stopping** solution to all wells at the same timed intervals as in step 6 (blue color turns yellow). Mix gently. Measure absorbance at 450 nm 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.

## DILUTION OF SAMPLES

Serum samples containing more than 50 ng/mL free beta-HCG must be diluted with the zero standard (standard A) and the results obtained should be multiplied by the appropriate dilution factor.

## Expected Values for free $\beta$ -HCG

### 1. hCG and Free $\beta$ -hCG Subunit Levels in Normal Pregnancy

A logarithmic increase in the serum concentration of hCG was observed from 5-8 weeks of gestation (2,600 ng/ml to 33,000 ng/ml) as defined by last menstrual period; thereafter, hCG values decreased. Similarly, free  $\beta$ -hCG levels increased rapidly to reach maximum levels (~60 ng/ml) at 8-9 weeks of pregnancy, followed by a gradual decline during the next 11-12 weeks of gestation.

At 5 weeks of gestation, the ratio of free  $\beta$ -hCG to intact hCG is approximately 1.0 % (w/w). Thereafter, this ratio remains remarkably constant over 22 weeks of gestation (~ 0.5 % w/w).

### 2. hCG and Free Subunits Levels in Gestational Choriocarcinoma

Free  $\alpha$  and free  $\beta$ -subunits and hCG levels were measured in five patients with untreated gestational choriocarcinoma. The concentrations in serum are shown in the following table:

Patient #	HCG (ng/ml)	$\alpha$ -HCG (ng/ml)	$\beta$ -HCG (ng/ml)
1	210,000	112	8000
2	22,195	20	1300
3	6,840	1	232
4	36,000	44	3900
5	4,200	2	350

The levels of free  $\alpha$ -hCG were low, ranging from 1-112 ng/ml, whereas hCG levels ranged from 4,200 to 210,000 ng/ml (1 ng ~ 15 mIU). In contrast, free  $\beta$ -hCG concentrations were found to be markedly elevated in choriocarcinoma.

## STANDARDIZATION

For intact hCG, 1 ng is approximately equivalent to 15 mIU (WHO, 1st IRP 75/537). For free  $\beta$ -hCG subunit, since there is no WHO standardization, we tested the free  $\beta$ -hCG against hCG ELISA kit, and found 1 ng of free  $\beta$ -hCG equals to 0.1 mIU in terms of hCG immunological activity.

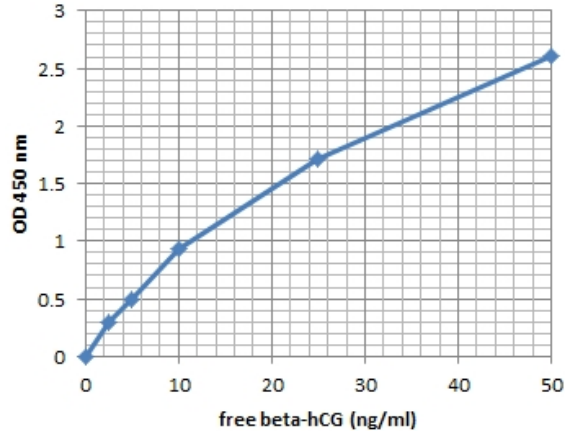
## Species Crossreactivity

This kit has been tested for human serum samples. The kit may be used in other biological fluids. It has not been tested in other species. For humans, the kit has been used in saliva, tissue lysate, and in mice expressing HCG-bearing tumors (see ADI publications on page 6).

## WORKSHEET OF TYPICAL ASSAY

Wells	Stds/samples	Mean A <sub>450nm</sub>
A1, A2	Std. A (0 ng/ml)	0.061
B1, B2	Std. B (2.5 ng/ml)	0.296
C1, C2	Std. C (5 ng/ml)	0.498
D1, D2	Std. D (10 ng/ml)	0.929
E1, E2	Std. E (25 ng/ml)	1.711
F1, F2	Std. F (50 ng/ml)	2.613

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

1. Calculate the mean absorbance value (A<sub>450</sub>) for each set of reference standards, controls and patient samples.
2. Construct a standard curve by plotting the mean absorbance obtained from each reference standard against its concentration in ng/ml on graph paper, with absorbance values on the vertical or Y axis, and concentrations on the horizontal or X axis.
3. Use the mean absorbance values for each specimen to determine the corresponding concentration of free Beta-hCG in ng/ml from the standard curve.

## PRINCIPLE OF THE TEST

Free  $\beta$ -HCG ELISA kit is based on sequential binding of Free  $\beta$ -HCG from patient samples to two antibodies, one immobilized on microtiter well plates, and other conjugated to the enzyme horseradish peroxidase. After a washing step, chromogenic substrate is added and color developed. The enzymatic reaction (color) is directly proportional to the amount of Free  $\beta$ -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  $\mu$ l) and Multichannel pipet with disposable plastic tips. Reagent troughs. Plate washer (recommended) and ELISA plate Reader.

## PRECAUTIONS

The Alpha Diagnostic International Free  $\beta$ -HCG ELISA kit is intended for *in vitro research* use only. The reagents contain proclin-300 as preservative; necessary care should be taken when disposing solutions. Some components have been tested using FDA-approved methods and has been found negative for antibody to human immuno-deficiency virus (HIV-I, HIV-II), antibody to Hepatitis C, and Hepatitis B surface antigen (HBsAg). No known test method can offer total assurance that HIV-I, HIV-II, hepatitis B and C virus or other infectious agents are absent. Handle these reagents as if they were potentially infectious. Information on handling human serum is provided in the CDC/NIH manual "Biosafety in Microbiological and Biomedical Laboratories" (U.S.A. HHS publication No.(NIH 88-8395.).

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

TMB (substrate), H<sub>2</sub>SO<sub>4</sub> (stop solution), and Proclin-300 (0.1% v/v in standards, sample diluent and HRP-conjugates).

## 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. Sodium azide, an inhibitor or peroxidase, must not be present in the samples.

### Preparation of the Reagents:

1. Reconstitute each Lyophilized Standard with 1 ml distilled water and gently mix for 10-15 min at room temp. Store at 4oC. Stable for 30-days at 2-4oC or stored frozen at -20oC for long-term.
2. Dilute wash buffer (100x) with distilled water (dilute 10-ml in 1-L water). Store at 4oC for 30-days/