

#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 Pregnenolone	#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)		

Human Growth Hormone (HGH)

ELISA KIT Cat. No. 1600

For Quantitative Determination of Human GH In Serum

For In Vitro Research Use Only



ALPHA DIAGNOSTIC
INTERNATIONAL

6203 Woodlake Center Drive • San Antonio • Texas 78244 • USA.

Phone (210) 561-9515 • Fax (210) 561-9544

Toll Free (800) 786-5777

Email: service@4adi.com

Web Site: www.4adi.com

Human Growth Hormone (HGH) HGH ELISA KIT Cat. No. 1600

For Quantitative Determination of Human GH In Serum

C o m p o n e n t s	C a t . N o .
Anti-human GH coated microwell strip plate (96 wells), Ready-to-use	1 6 0 1
HGH Standard A (ready-to-use), 2 ml	# 1 6 0 2 A
HGH Standard B (ready-to-use), 0.5 ml	# 1 6 0 2 B
HGH Standard C (ready-to-use), 0.5 ml	# 1 6 0 2 C
HGH Standard D (ready-to-use), 0.5 ml	# 1 6 0 2 D
HGH Standard E (ready-to-use), 0.5 ml	# 1 6 0 2 E
HGH Standard F (ready-to-use), 0.5 ml	# 1 6 0 2 F
HGH Low Control (ready-to-use), 0.5 ml	# 1 6 0 2 L C
HGH High Control (ready-to-use), 0.5 ml	# 1 6 0 2 H C
Anti-hGH HRP Conjugate (100X) , 200 ul	# 1 6 0 3
Assay Buffer , 15 ml	# 1 6 0 4
HRP substrate; Solution (ready-to-use), 16 ml	# 1 6 0 0 T M B
Wash buffer (10X), 50 ml, dilute 1:10 with distilled water,	W - 1 0
Stop solution (ready-to-use), 6 ml	T - 1 0
Complete Instruction Manual	M - 1 6 0 0

Human growth hormone (GH, Mr ~22 kDa) is a single polypeptide chain consisting of 191 amino acid residues and contains two intrachain disulfide bonds. GH is secreted by the somatotrophs, which constitute approximately 50% of the anterior pituitary cells. The structure of growth hormone is very similar to that of human placental lactogen; there is 92% homology between the two structures.

Growth hormone is necessary for normal linear growth. Its deficiency causes short stature while excess (prior to epiphyseal closure) leads to gigantism. The effects of growth hormone on skeletal growth are mediated by stimulation of somatomedin. Serum growth hormone levels are undetectable much of the day; levels peak after meals and undergo a sustained rise during sleep. This pulsatile nature of growth hormone secretion must be taken into consideration when determining GH levels in a clinical situation. Normal (fasting) growth hormone levels are usually considered to be less than 10 ng/ml. However, most bedridden patients have much lower concentrations of the growth hormone (approximately 1 ng/ml), whereas ambulatory patients are likely to show higher levels of GH, up to 10 ng/ml. This could be due to the fact that exercise stimulates growth hormone secretion.

PERFORMANCE CHARACTERISTICS

1. DETECTION LIMIT

Based on twenty replicates determinations of the zero standard, the minimum concentration of human GH detected using this assay is 0.3 ng/ml. The detection limit is defined as the value deviating by 2 SD from the zero standard.

2. PRECISION

INTRA-ASSAY PRECISION:

Three samples were assayed ten times each on the same calibrator curve. The results (in ng/ml) are tabulated below:

Sample	Mean	SD	CV%
1	1.46	0.09	5.8
2	12.33	0.68	5.5
3	41.87	0.97	2.3

INTER-ASSAY PRECISION:

Three samples were assayed ten times over a period of four weeks. The results (in ng/ml) are tabulated below:

Sample	Mean	SD	CV%
1	2.95	0.27	9.0
2	19.29	0.86	4.4
3	36.06	1.72	4.7

3. RECOVERY

A known amount of hGH (2.5, 10, and 50 ng/ml) was added to three patient sera (with original GH concentrations of 0.9 ng/ml) and the total HGH concentrations measured. The assay showed excellent mean recoveries of about 97% (range 94-98%).

4. LINEARITY

A patient sample (with an original HGH concentration of 25 ng/ml) was diluted (1:4, 1:8, 1:16, 1:32, and 1:64) with the zero standard and the final HGH values determined. The sample showed excellent mean recoveries of about 94% (range 92-109%).

5. SPECIFICITY

The specificity of HGH kit was determined by measuring interference from high concentrations of HCG (up to 2000 mIU/ml) and human prolactin (up to 200 ng/ml). No significant cross reactivity was observed at these concentrations.

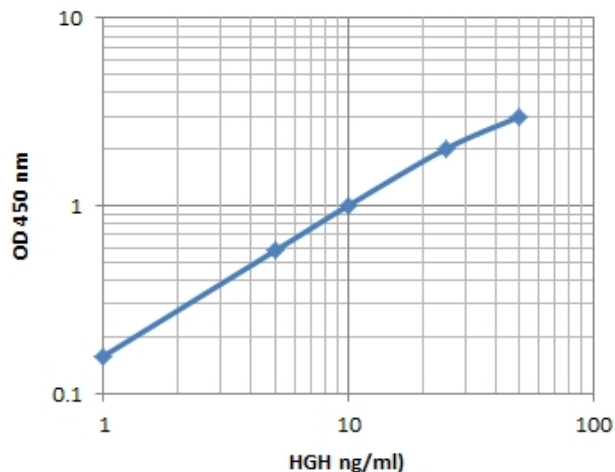
6. Sensitivity:

The sensitivity of the HGH elisa kit is **0.2 ng/ml**

WORKSHEET OF TYPICAL ASSAY

Wells	Stds/samples	Mean A _{450 nm}	Calculated Conc. (ng/ml)
A1, A2	Std. A (0 ng/ml)	0.073	
B1, B2	Std. B (1 ng/ml)	0.159	
C1, C2	Std. C (5 ng/ml)	0.580	
D1, D2	Std. D (10 ng/ml)	1.014	
E1, E2	Std. E (25 ng/ml)	2.009	
F1, F2	Std. F (50 ng/ml)	2.773	
G1, G2	Sample 1	0.555	

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-specs-XL

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

PRINCIPLE OF THE TEST

HGH ELISA kit is based on simultaneous binding of human Growth Hormone (HGH) from 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 HGH present in the sample. The reaction is terminated by adding stopping solution. Absorbance is then measured on a microtiter well ELISA reader at 450 nm. and the concentration of HGH in samples and control is 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 HGH ELISA test is intended for *in vitro research* use only. The reagents contain thimerosal as preservative; necessary care should be taken when disposing solutions. The Control Serum has 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 Prolcin-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.

Preparation of reagents:

Mouse Anti-hGH HRP conjugate conc.: Dilute 1:100 in assay buffer before use (eg; 20 ul of HRP in 2 ml of Assay buffer)

Wash buffer is supplied as 10x stock. Dilute 50 ml into 450 ml de-ionized or distilled water, mix, and store at room temp for 1-2 weeks. It can be stored at 4°C for long term storage.

REAGENTS INCLUDED IN THE KIT

Microtiter 96-well strip plate coated with mouse anti-hGH antibody
HGH Standards. Ready-to-use. Standard A-F, 0.5 ml

Anti-hGH HRP Conjugate conc., 200 ul, **Dilute 1:100 in assay buffer**

HRP Substrate, Solution , 16 ml. Ready for use.

Stop Solution, 6 ml. Ready for use.

Wash buffer (10X), 50 ml, **Dilute 50 ml into 450 ml de-ionized water**

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 frozen in suitable aliquots for long-term use. Repeated freezing and thawing is not recommended.

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

Dilute wash buffer (1:10) with distilled water (10 ml stock in 450 ml distilled water).

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. Add **100 µl** of enzyme conjugate into **each well**. Mix gently.
4. Cover the plate and incubate on a plate shaker(approx. 200 rpm) for **60 minutes** at room temperature.
5. Aspirate and wash the wells **3 times** with wash buffer (300 µl/wash). 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. Dispense **100 ul TMB substrate per well**. Mix gently.
7. Cover the plate and incubate on a plate shaker for **10-15 minutes** at room temperature.
8. Stop the reaction by adding **50 µl** of stopping solution to **all wells** at the same timed intervals as in step 6. Mix gently.
9. Measure the absorbance at 450 nm using an ELISA reader.

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 five 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.

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 HGH concentrations. Read off the HGH concentrations of the control and patient samples.

EXPECTED VALUES

It is recommended that each laboratory must determine its own normal and abnormal ranges.