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
#100-600-IGF	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-100-600-IGF

Human Insulin-like growth factor I (IGF-1)

ELISA KIT Cat. # 100-600-IGF, 96 Tests

For Quantitative Determination of IGF-1 In serum, plasma, and cell culture supernatants

This kit is for in research use only (RUO) and not for diagnostic, therapeutic or prevention of the disease.



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Human IGF-1 ELISA KIT # 100-600-IGF

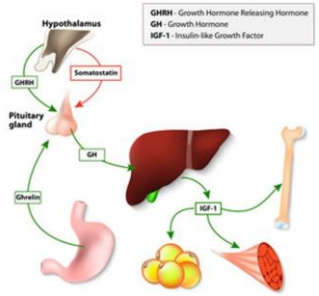
Kit Contents: (reagents for 96 tests)

Components	96 tests
IGF-1 antibody **coated strip plate** (96 wells), #100601	1 Plate
IGF-1 **Std Protein** (**lyophilized** powder, 100 ng/vial), #100600A	2 vials
Biotinylated anti-human IGF1 **Detection antibody** (**lyophilized**), #100602	2 vials
Assay diluent, 30mL, #100603	2 bottles
HRP-Strep conjugate (120X), 0.2mL, #100604	1 vial
TMB Substrate Solution, 12 ml, # 100600-TMB	1 bottle
Wash Buffer (20X); 25 ml, , #100600-W B	1 bottle
Stop solution, 8 ml, # 100600-ST	1 bottle
Instruction Manual, M - 1 0 0 - 6 0 0 - I G F	1

Intended Use

ADI's Human Insulin IGF-1 kit is a sandwich ELISA for the detection and measurement of IGF-1 in human serum, plasma or other biological fluids (culture medium etc). For research use only (RUO), not for diagnosis, cure or prevention of the disease.

General information



The insulin-like growth factors (IGFs) are part of a complex system, the IGF "axis", consists of two cell-surface receptors (**IGF1R and IGF2R**), two ligands (Insulin-like growth factor 1 (**IGF-I**) and Insulin-like growth factor 2 (**IGF-2**)), a family of six high-affinity IGF-binding proteins (**IGFBP-1 to IGFBP-6**), as well as associated IGFBP degrading enzymes, referred to collectively as **proteases**. The IGF "axis" is also referred to as the Growth Hormone/IGF-I Axis. IGF-1) is mainly secreted by the liver as a result of stimulation by growth hormone (GH). IGF-I is important for both the regulation of normal physiology, as well as a number of pathological states, including cancer. Insulin-like growth factor 2 (IGF-2) is thought to be a primary growth factor required for early development while IGF-1 expression is required for achieving maximal growth. Factors that are thought to cause variation in the levels of GH and IGF-1 in the circulation include an individual's genetic make-up, the time of day, age, sex, exercise status, stress levels, nutrition level, body mass index (BMI), disease state, race, estrogen status, and xenobiotic intake.

PERFORMANCE CHARACTERISTICS

SENSITIVITY

The minimum detectable dose of Human IGF-1 was determined to be **100 pg/ml**. Minimum detectable dose is defined as the analyte concentration resulting in an absorbance that is 2 standard deviations higher than that of the blank (diluent buffer)

SPIKING & RECOVERY

Recovery was determined by spiking various levels of Human IGF-1 into the sample types listed below. Mean recoveries are as follows:

Sample type	Average % recovery	Range %
Serum	119.6	105-138
Plasma	105.4	81-121
Cell culture media	102.5	98-110

LINEARITY

The results of IGF-1 concentration in the value of ng/mL are as follows:

Sample	Type	Serum	Plasma	Cell culture media
1:2	Average % expected range %	116.4 108-124	111.9 107-125	103.3 94-118
1:4	Average % expected range %	121.9 114-130	104.6 81-120	84.62 77-92

REPRODUCIBILITY

Intra-Assay CV%: <10% Inter-Assay CV%: <12%

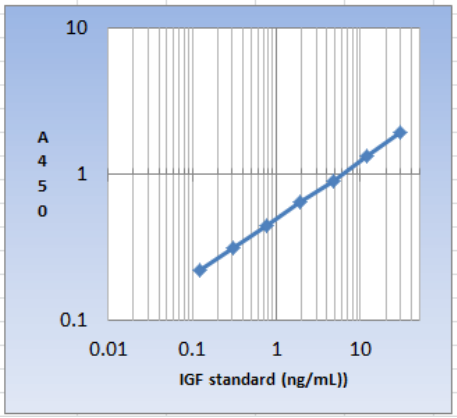
SPECIFICITY

This IGF-1 ELISA kit shows no cross-reactivity with any of the cytokines tested: Human Angiogenin, BDNF, BLC, ENA-78, FGF-4, IGFBP-1, IGFBP-2, IGFBP-3, IGFBP-4, IL-1 alpha, IL-1 beta, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL11, IL-12 p70, IL-12 p40, IL-13, IL-15, I-309, IP-10, G-CSF, GM-CSF, IFN-gamma, Leptin (OB), MCP-1, MCP-3, MDC, MIP-1 alpha, MIP-1 beta, MIP-1 delta, PARC, RANTES, SCF, TARC, TGF-beta, TIMP-1, TIMP-2, TNF-alpha, TNF-beta, TPO, VEGF.

WORKSHEET OF TYPICAL ASSAY

Wells	Stds/samples	A450nm
A1, A2	(0 ng/mL)	0.115
B1, B2	(0.123 ng/mL)	0.220
C1, C2	(0.307 ng/mL)	0.311
D1, D2	(0.768 ng/mL)	0.443
E1, E2	(1.92 ng/mL)	0.644
F1, F2	(4.8 ng/mL)	0.899
G1, G2	(12 ng/mL)	1.314
H1, H2	(30 ng/mL)	1.945

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. If ELISA reader software is being used, we recommend 4-paramter or 5-parameter curve.



8_ADI_ELISA_kits_Graphs_160124A

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

INTERPRETATION OF RESULTS

Calculate the mean absorbance for each set of duplicate standards, controls and samples, and subtract the average zero standard optical density. Plot the standard curve on **log-log graph** paper or using Sigma plot software, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit straight line through the standard points. If using a graph or calculation program, we recommend using 4-poin-logistic curve for sample calculations.

IGF-I has an involvement in regulating neural development. Increased serum levels of IGF-I in children have been associated with higher IQ. IGF-I shapes the development of the cochlea through controlling apoptosis. Its deficit can cause hearing loss. Serum level of it also underlies a correlation between short height and reduced hearing abilities particularly around 3–5 years of age, and at age 18 (late puberty). IGF-1 exert its function primarily thru the IGF1R. Since many distinct tissue types express the IGF-1 receptor, IGF-1's effects are diverse. IGF-1 and IGF-2 are regulated by a family of proteins known as the IGF-Binding Proteins.

Insulin-like growth factor 1 (IGF-1), also called somatomedin C, is a protein that in humans is encoded by the IGF1 gene. IGF-1 (70-aa, ~7.6 kda) is a hormone similar in molecular structure to insulin. A synthetic analog of IGF-1, mecasermin (brand name Increlex), is used for the treatment of growth failure. GF-1 levels can be measured in the blood in 10-1000 ng/ml amounts. IGF-1 is used by physicians as a screening test for growth hormone deficiency and excess in acromegaly and gigantism. IGF-1 is used by physicians as a screening test for growth hormone deficiency and excess in acromegaly and gigantism. Sequential management over time is often useful for the management of several types of pituitary disease, undernutrition, and growth problems.

IGF-1 analogs: Long arginine 3-IGF-1 (IGF-1 LR3 or as LR3-IGF-1) is a synthetic protein (R3E, and an additional 13-aa at the N-terminus, 83-aa, T1/2=20-30 hrs Vs IGF-1 12-15 hrs). **des(1-3)IGF-1** is a naturally-occurring, endogenous protein (missing 1-3 aa, 67-aa, reduced binding to IGFBPs, ~10-fold more potent than IGF-1). Human IGF-1 (70-aa) is quite conserved in various species: Horse and camel (94%), rabbit (93%), cow, pig, cat (91%), canine, goat (90%), and mouse (83%).

PRINCIPLE OF THE TEST

Human IGF-1 is a sandwich ELISA for the quantitative measurement of human IGF-1 in serum, plasma, and cell culture supernatants (free form). It can be adapted to measure total IGF-1 with the optional extraction protocol. This assay employs an antibody specific for human IGF-1 coated on a 96-well plate. Standards and samples are pipetted into the wells and IGF-1 present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti-human IGF-1 antibody is added. After washing away unbound biotinylated antibody, HRP- conjugated streptavidin is pipetted to the wells. The wells are again washed, a TMB substrate solution is added to the wells and color develops in proportion to the amount of IGF-1 bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm

MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipet (25-100 µl) and Multichannel pipet with disposable plastic tips. Beakers, DI water. Reagent troughs, Plate washer (recommended) and ELISA plate Reader.

PRECAUTIONS

IGF-1 ELISA test is intended for *in vitro research* use only (RUO). Wear gloves while performing this assay and handle these reagents as if they are potential infectious. Avoid contact with reagents containing TMB, hydrogen peroxide, or sulfuric acid. Use Good Laboratory Practices. 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).

http://4adi.com/commerce/info/showpage.jsp?page_id=1060&category_id=2430&visit=10

STORAGE AND STABILITY

The microtiter well plate and all other reagents are stable at 2-8^{o}C until 6 months. The unused portions of the standards can be frozen in suitable aliquots for long-term use. Repeated freezing and thawing is not recommended.

Sample dilution:

Assay Diluent should be used for dilution of serum, plasma, and cell culture supernatant samples. The suggested dilution for normal **serum/plasma is 2 - 20 fold**.
Note: Levels of IGF-1 may vary between different samples. Optimal dilution factors for each sample must be determined by the investigator.

Preparation of standards:

Briefly spin a vial of lyophilized stock standard. Add 400 µl Assay Diluent into vial to prepare a **100 ng/ml standard**. Follow the table to prepare the rest of the standards. Do not keep the diluted standards beyond the assay date and prepare only in required amounts.

	Volume	Assay diluent	Stds name	Final Concn
Stock Std (100 ng/ml)	150 µl	350 µl	Std. G	30 ng/ml
Std. G	200 µl	300 µl	Std. F	12 ng/ml
Std. F	200 µl	300 µl	Std. E	4.8 ng/ml
Std. E	200 µl	300 µl	Std. D	1.92ng/ml
Std. D	200 µl	300 µl	Std. C	0.768 ng/ml
Std. C	200 µl	300 µl	Std. B	0.307 ng/ml
Std. B	200 µl	300 µl	Std. A	0.123 ng/ml
Blanks	0	300 µl	Blanks	0 ng/ml

REAGENT PREPARATION

1. Bring all reagents and samples to room temperature (18 - 25ºC) before use.

2. **1X wash buffer**- Dilute **Wash Concentrate (20X)** 1:20 with water (**20 ml of stock into 400 ml water**). If stock contains visible crystals, warm to room temperature and mix gently until dissolved. Store at 4oC used until used.

3. **1X Detection Antibody** vial supplied as powder. **Add 100 µl of Assay Diluent to 1 of the lyophilized vial**. Mix gently to dissolve to prepare **80X stock**. Store at 4oC until used or up to 5 days. The 80X-detection antibody is diluted 1:80 with Assay Diluent (10 ul of stock antibody in 790 ul of diluent (need 100 ul/well or 10 ml for full plate).

4. **1X HRP-Streptavidin concentrate** via supplied as 120X liquid stock. It should be diluted 1:120 with the assay diluent prior to use. (10 ul of stock conjugate in 1.2 ml of diluent (need 100 ul/well; add 100 ul into 12 ml of diluent for full plate). Store at 4oC until used or up to 5 days. Prepare only in required amounts.

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.

1. Label or mark the microtiter well strips to be used on the plate.

2. Pipet **100 µl sample and standards**, into appropriate wells in *duplicate*.

3. Mix plate by placing on a **plate shaker at 400-450 RPM** for 30 seconds. Cover the plate and incubate for **2.5 hrs** at room temperature (18-25 oC) or overnight at 4ºC with gentle shaking.

4. Aspirate and **wash the wells 4 times** with 300 µl of diluted 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. Add **100 µl of 1X biotinylated (detection) antibody** into each well.

6. Mix plate by placing on a **plate shaker at 400-450 RPM** for 30 seconds. Cover the plate and incubate for **60 minutes** at room temp. (18-25oC). Aspirate and **wash the wells 4 times** with 300 µl of 1X wash buffer.

7. Add **100 µl** of **1X Streptavidin conjugate** to each well. Cover the plate and incubate for **45 minutes** at room temp. (18-25 oC) with gentle shaking. Aspirate and **wash the wells 4 times** with 300 µl of 1X wash buffer.

8. Dispense **100 ul TMB substrate per well.** Mix gently. Cover the plate and incubate for **30 minutes** at room temp with gentle shaking (standards and positive wells develop blue color).

9. Immediately add **50 µL** of ELISA **Stop Solution** into each of the wells. Mix gently (blue color turns yellow). Read the absorbance at 450 nm.

NOTES: Read instructions carefully before the assay. Do not allow reagents to dry on the wells. 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. 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. Plate readers measure absorbance vertically. Do not touch the bottom of the wells.

References: Jansen M (1983) Nature 306, 609-611; Rinderknecht E (1978) JBC 253, 2769-2776; Scarth JP (2006) xenobiotica 36, 119-218; Rosenbloom AL (2007) Curr. Opinion. Pediat. 19, 458-464; Arnaledez FI (2012) Hematology/Oncology Clinics of North America 26 (3): 527–42; Delafontaine P (2005) Arterioscler. Thromb. Vasc. Biol. 24 (3): 435–44; Sandhu MS (205)