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)		
#3410	Human serum Neopterin		

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

Instruction Manual No. 6430-30-M

Human interleulin-6 (hIL-6)

ELISA KIT Cat. No. 6430-30

For Quantitative Determination of human IL-6 In Human Serum/Plasma/Culture medium

For In Vitro Research Use Only

ALPHA DIAGNOSTIC INTERNATIONAL

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DRAFT MANUAL: PLEASE CONSULT THE MANUAL SUPPLIED WITH THE KIT FOR ANY LOT SPECIFIC CHNAGES.

Human IL-6 ELISA KIT Cat. No. 6430-30 (96 tests)

Kit Components (96 tests)	#
Anti-hIL-6 IgG coated plate, (96 wells) 6430301	1 plate
Rec. hIL-6 **Std.** 10 ng/vial #6430302A, lyophilized.	2 vials
Biotinylated Anti-hIL-6 IgG **(100X);** 130 ul, #6430303	1 vial
Avidin-Biotin-Peroxidase Complex (ABC), **100X,** 130 ul #6430304	1 vial
Wash buffer powder, #643030WB (dissolve in 2-L water)	1 bottle
Sample diluent buffer: 30 ml, #6430305	1 bottle
Antibody Diluent buffer: 12 ml, #6430306	1 bottle
ABC Diluent buffer: 12 ml, #6430307	1 bottle
TMB substrate: 10ml, #643030TMB	1 bottle
TMB **stop solution**: 10ml #643030SS	1 bottle
Complete Instruction Manual # M-6430-30	1

Interleukin-6 (IL-6) is an interleukin that acts as both a pro-inflammatory and anti-inflammatory cytokine. In humans, it is encoded by the IL6 gene. IL-6 is secreted by T cells and macrophages to stimulate immune response, e.g. during infection and after trauma, especially burns or other tissue damage leading to inflammation. IL-6 also plays a role in fighting infection, as IL-6 has been shown in mice to be required for resistance against bacterium Streptococcus pneumoniae. IL-6 is also considered a "myokine," a cytokine produced from muscle, and is elevated in response to muscle contraction. It is significantly elevated with exercise, and precedes the appearance of other cytokines in the circulation. During exercise, it is thought to act in a hormone-like manner to mobilize extracellular substrates and/or augment substrate delivery. Additionally, osteoblasts secrete IL-6 to stimulate osteoclast formation. Smooth muscle cells in the tunica media of many blood vessels also produce IL-6 as a pro-inflammatory cytokine. IL-6's role as an anti-inflammatory cytokine is mediated through its inhibitory effects on TNF-alpha and IL-1, and activation of IL-1ra and IL-10. Interleukin-6 (IL6) has come to be regarded as a potential osteoporotic factor because it has stimulatory effects on cells of the osteoclast lineage, and, thus, may play a role in the pathogenesis of bone loss associated with estrogen deficiency.2 IL-6 has many roles essential to the regulation of the immune response, haematopoiesis, and bone resorption.3 It is involved not only in the hepatic acute phase response but also in adipose tissue metabolism, lipoprotein lipase activity, and hepatic triglyceride secretion. Overproduction of IL-6, a proinflammatory cytokine, is associated with a spectrum of age-related conditions including cardiovascular disease, osteoporosis, arthritis, type 2 diabetes, certain cancers, periodontal disease, frailty, and functional decline. BSF-2 is a novel interleukin consisting of184 amino acids.

ADi's hIL-6 ELISA kits is for detection of human IL-6 in sera, plasma, body fluids, tissue lysates or cell culture supernatants. For in vitro research use only.

Quality Control

Good laboratory practice requires that controls be run with each calibration curve. A statistically significant number of controls should be assayed to establish mean values and acceptable ranges to assure proper performance. It is recommended to use control samples according to state and federal regulations. The use of control samples is advised to assure the day to day validity of results. Use controls at both normal and pathological levels. It is also recommended to make use of national or international Quality Assessment programs in order to ensure the accuracy of the results. Employ appropriate statistical methods for analysing control values and trends. If the results of the assay do not fit to the established acceptable ranges of control materials patient results should be considered invalid. In this case, please check the following technical areas: Pipetting and timing devices; photometer, expiration dates of reagents, storage and incubation conditions, aspiration and washing methods. After checking the above mentioned items without finding any error contact your distributor or ADI directly.

PERFORMANCE CHARACTERISTICS

Range
4.69pg/ml-300pg/ml

Sensitivity

< 0.3pg/ml

Specificity

No detectable cross-reactivity with any other cytokine.

Reliability of Results

The test must be performed exactly as per the manufacturer's instructions for use. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable national standards and/or laws. This is especially relevant for the use of control reagents. It is important to always include, within the test procedure, a sufficient number of controls for validating the accuracy and precision of the test. The test results are valid only if all controls are within the specified ranges and if all other test parameters are also within the given assay specifications. In case of any doubt or concern please contact ADI.

Species reactivity

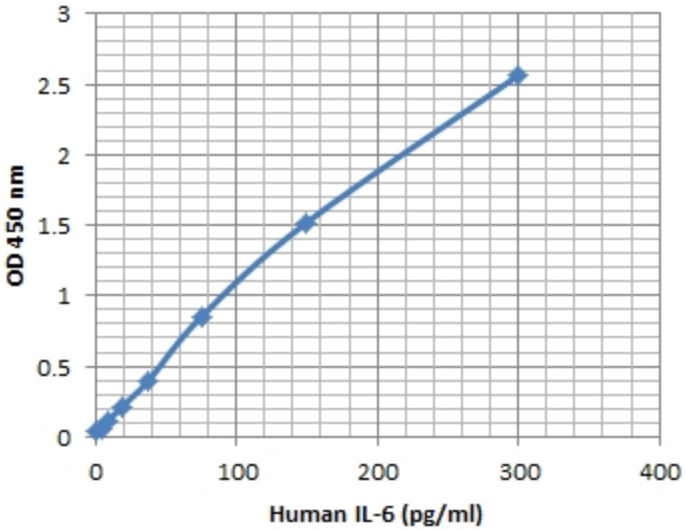
This kit has been designed and tested for human samples only. It has not been tested in animals (rat, mouse, etc). ADI has separate kits for mouse and rat IL-6.

Refs: Bowock AM (1988) Genmcis 3, 8-16; Kiecolt-Glaser, J. K (2003) PNAS 100, 9090-9095; Hirano T (1986) Nature 324, 73-76

WORKSHEET OF A TYPICAL ASSAY

Wells	Stds/samples	Mean A450
A1, A2	blanks	0.010
B1, B2	**Std. A** (4.69 pg/ml)	0.057
C1, C2	**Std. B** (9.38 pg/ml)	0.116
D1, D2	**Std. C** (18.75 pg/ml)	0.215
E1, E2	**Std. D** (37.5 pg/ml)	0.398
F1, F2	**Std. E** (75 pg/ml)	0.851
G1, G2	**Std. F** (150 pg/ml)	1.522
B3, B4	**Std. G** (300 pg/ml)	2.565
Sample 1		1.49

NOTE: These data are for demonstration purpose only.



**Kit-spec-XL*

CALCULATION OF RESULTS

For calculation, (the relative O.D.450) = (the O.D.450of each well) – (the O.D.of Zero well). The standard curve can be plotted as the relative O.D450 of each standard solution (Y) vs. the respective concentration of the standard solution (X). The human IL-6 concentration of the samples can be interpolated from the standard curve. **Note:** if the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.

PRINCIPLE OF THE TEST

The ADI human IL-6 ELISA Kit was based on standard sandwich ELISA. Human IL-6 specific monoclonal antibodies were precoated onto 96-well plates. The human specific detection polyclonal antibodies were biotinylated. The test samples and biotinylated detection antibodies were added to the wells subsequently and then followed by washing with PBS or TBS buffer. Avidin-Biotin-Peroxidase Complex was added and unbound conjugates were washed away with PBS or TBS buffer. HRP substrate TMB was used to visualize HRP enzymatic reaction. TMB was catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. The density of yellow is proportional to the human IL-6 amount of sample captured in plate.

MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipet (25-100 μl) and multichannel pipet with disposable plastic tips. Reagent troughs, Orbital shaker, plate washer (recommended) and ELISA plate Reader.

PRECAUTIONS

ADI hIL-6 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), Diluted H2SO4 (1N, stop solution), and Thimerosal (0.02% v/v in standards, conjuagte diluent and HRP-conjugates).

Sample Preparation and Storage

Store samples to be assayed within 24 hours at 2-8°C. For long-term storage, aliquot and freeze samples at -20°C. Avoid repeated freeze-thaw cycles.

o **Cell culture supernate, tissue lysate or body fluids**: Remove particulates by centrifugation, analyze immediately or aliquot and store at -20°C

o **Serum**: Allow the serum to clot in a serum separator tube (about 30 min) at room temperature. Centrifuge at approximately 1000 X g for 15 min. Analyze the serum immediately or aliquot and store frozen at -20°C.

o **Plasma**: Collect plasma using heparin, EDTA, citrate as an anticoagulant. Centrifuge for 15 min at 1000 x g within 30 min of collection. Analyze immediately or aliquot and store frozen at -20°C.

Sample Dilution Guideline

The user needs to estimate the concentration of the target protein in the sample and select a proper dilution factor so that the diluted target protein concentration falls near the middle of the linear regime in the standard curve. Dilute the sample using the provided diluent buffer. The following is a guideline for sample dilution. Several trials may be necessary in practice. **The sample must be well mixed with the diluents buffer.**

o **High target protein concentration (3-30ng/ml).** The working dilution is 1:100. i.e. Add 1 µl sample into 99 µl sample diluent buffer.

o **Medium target protein concentration (0.3-3ng/**ml**).** The working dilution is 1:10. i.e. Add 10 µl sample into 90 µl sample diluent buffer.

o **Low target protein concentration (4.69-300pg/ml).** The working dilution is 1:2. i.e. Add 50 µl sample to 50 µl sample diluent buffer.

o Very Low target protein concentration (=4.69pg/ml). No dilution necessary, or the working dilution is 1:2.

Reagent Preparation

1. **Reconstitution of the human IL-6 standard**: IL-6 standard solution should be prepared no more than 2 hours prior to the experiment. Two vials of IL-6 standard (10ng per tube) are included in each kit. Use one tube for each experiment.

a. 10,000pg/ml of human IL-6 standard solution: Add 1 ml sample diluent buffer into one tube, keep the tube at room temperature for 10 min and mix thoroughly.
b. 300pg/ml of human IL-6 standard solution: Add 0.03 ml of the above 10ng/ml IL-6 standard solution into 0.97 ml sample diluent buffer and mix thoroughly.
c. 150pg/ml→4.69pg/ml of human IL-6 standard solutions: Label 6 Eppendorf tubes with 150pg/ml, 75pg/ml, 37.5pg/ml, 18.75pg/ml, 9.38pg/ml, 4.69pg/ml, respectively. Aliquot 0.3 ml of the sample diluent buffer into each tube. Add 0.3 ml of the above 300pg/ml IL-6 standard solution into 1st tube and mix. Transfer 0.3 ml from 1st tube to 2nd tube and mix. Transfer 0.3 ml from 2nd tube to 3rd tube and mix, and so on.

Note: The standard solutions are best used within 2 hours. The 10ng/ml standard solution may be stored at 4°C for up to 12 hours, or at -20°C for up to 48 hours. Avoid repeated freeze thaw cycles.

2. **Preparation of biotinylated anti-human IL-6 antibody working solution**: The solution should be prepared no more than 2 hours prior to the experiment.

a. The total volume should be: 0.1ml/well x (the number of wells). (Allowing 0.1-0.2 ml more than total volume)
b. Biotinylated anti-human IL-6 antibody should be diluted in 1:99 with the antibody diluent buffer and mixed thoroughly.

3. **Preparation of Avidin-Biotin-Peroxidase Complex (ABC) working solution**: The solution should be prepared no more than 1 hour prior to the experiment.

a. The total volume should be: 0.1ml/well x (the number of wells). (Allowing 0.1-0.2 ml more than total volume)
b. b. Avidin-Biotin-Peroxidase Complex (ABC) should be diluted in 1:99 with the ABC dilution buffer and mixed thoroughly.

4. **Prepare wash buffer** by adding the contents of the packet to a 2 liter container with one liter of distilled water. Use a stir plate to completely dissolve, and then adjust the total volume to 2 liters. Stir after final volume adjustment. Wash buffer solution is stable at 2-8°C for 30 days.

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 at 2-8oC. The unused ELISA kits components (Standards, Biotin-antibody, and ABC conjugates) can be stored at -20oC for up to 12 months. Rest of the kit components including the coated plates can be stored at 4oC for up to 12 months. Do not freeze and thaw repeatedly.

TEST PROCEDURE.

The ABC working solution and TMB color developing agent must be kept warm at 37°C for 30 min before use. When diluting samples and reagents, they must be mixed completely and evenly. Standard IL6 detection curve should be prepared for each experiment. The user will decide sample dilution fold by crude estimation of IL-6 amount in samples.

1. **Add 0.1 ml per well of the human IL-6 standard solutions** (300pg/ml, 150pg/ml, 75pg/ml, 37.5pg/ml, 18.75pg/ml, 9.38pg/ml, 4.69pg/ml) into the precoated 96-well plate. Add 0.1 ml of the sample diluent buffer into the control well (Zero well). **Add 0.1ml of each properly diluted sample** of human sera, plasma, body fluids, tissue lysates or cell culture supernatants to each empty well. **See "Sample Dilution Guideline" above for details.** We recommend that each human IL-6 standard solution and each sample is measured in duplicate.

2. Seal the plate with the cover and **incubate at 37°C for 90 min**.

3. **Remove the cover, discard plate content**, and blot the plate onto paper towels or other absorbent material. Do NOT let the wells completely dry at any time.

4. **Add 0.1ml of biotinylated anti-human IL-6 antibody** working solution into each well and incubate the plate at **37°C for 60 min**.

5. **Wash the plate three times** with wash buffer, and each time let washing buffer stay in the wells for 1-2 min. Discard the washing buffer and blot the plate onto paper towels or other absorbent material.

6. **Add 0.1 ml of prepared ABC working solution** into each well and incubate the plate at **37°C for 30 min**.

7. **Wash plate 5 times with wash buffer** as in step 5. . Discard the washing buffer and blot the plate onto paper towels or other absorbent material.

8. **Add 90 µl of prepared TMB color** developing agent into each well and incubate plate at **37°C for 25-30 min** (shades of blue can be seen in the wells with the four most concentrated human IL-6 standard solutions; the other wells show no obvious color).

9. **Add 0.1 ml of prepared TMB stop solution** into each well. The color changes into yellow immediately.

10. **Read the O.D. absorbance at 450nm** in a microplate reader within 30 min after adding the stop solution.