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-0010

Human Leptin ELISA KIT

Cat. No. 0010, 96 Tests

For Quantitative Determination of Leptin In Human Serum

For In Vitro Research Use Only

ALPHA DIAGNOSTIC INTERNATIONAL

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LIFE TECH Life Technologies™
Your Molecular & Cell Technology Partner

Human Leptin ELISA KIT Cat. # 0010

Kit Components	96 tests
Anti-Human Leptin coated strip plate (96 wells), Cat. # 0 0 1 1	1 plate
Human Leptin **Std. A** (**0 ng/ml**); 0.5 ml, Cat # 0012A	1 Vial
Human Leptin **Std. B** (**1 ng/ml**); 0.5 ml, Cat # 0012B	1 Vial
Human Leptin **Std. C** (**5 ng/ml**); 0.5 ml, Cat # 0012C	1 Vial
Human Leptin **Std. D** (**10 ng/ml**); 0.5 ml, Cat # 0012D	1 Vial
Human Leptin **Std. E** (**20 ng/ml**); 0.5 ml, Cat # 0012E	1 Vial
Human Leptin **Std. F** (**50 ng/ml**); 0.5 ml, Cat # 0012F	1 Vial
Human Leptin **Std. G** (**100 ng/ml**); 0.5 ml, Cat # 0012G	1 Vial
Human Leptin **Controls**; **0.5 ml** **Low & High** (see lot sp. conc on the vials)	2 Vials
Anti-Leptin-Biotin Conjugate, **10 ml,** Cat # 0014	1 Vial
Streptavidin-HRP Conjugate (50X), **0.40 ml,** Cat # 0015 (before use dilute 1:50 with assay buffer)	1 Vial
Assay buffer, **20 ml,** Cat # 0016	1 Vial
Wash buffer concn. (**10X** solution) , Cat. # W - 5 0	50 ml
TMB substrate, Cat. # S - 5 0	16 ml
Stop solution, Cat. # T - 5 0	6 ml
Instruction Manual, M - 0 0 1 0	1

Intended Use

Leptin ELISA kit is a sandwich assay for the measurement of leptin human serum, plasma or other biological fluids. For in vitro research use only (RUO).

Introduction

Obesity, a common nutritional disorder, is associated with diabetes, hypertension, hyperlipidemia, cancer and many other health related problems. At least five genes, Obese (ob), diabetes (db), fat (fat), agouti yellow (A^y), and tubby (tub) have been linked to obesity. Recently, Ob genes (mouse and human) have been cloned. Obese gene encodes an adipocyte-tissue derived secreted **Ob protein/Leptin** (167 amino acid, ~16 kDa) that controls body weight homeostasis. Exogenous administration of recombinant Ob protein can reduce food intake and body weight. However, Ob protein had no effect in db/db mice suggesting a defect in leptin signaling mechanism. Leptin exert its effects by interacting with leptin receptors (obese receptors). The Ob-R has been shown to be a product of db gene that has long been thought to encode the receptor for a weight-controlling hormone. The Ob-R has at least 6 alternatively spliced forms with modifications at the amino and C-terminus.

3. RECOVERY

A known amount of leptin (12, 6, and 3 ng/ml) was added to two patient sera (with original leptin concentrations of 10 and 5.5 ng/ml) and the total leptin concentration measured. The assay showed excellent mean recoveries of about 105% (range 98-116%).

4. LINEARITY

Two patient samples (with original leptin concentration of. 34 and 85 ng/ml were diluted (1:2, 1:4, 1:8, and 1:16) and their final leptin values determined. The samples showed excellent mean recoveries of about 96%, and 95%, respectively (range 86-117%).

5. SPECIFICITY

The specificity of leptin ELISA kit was determined by measuring interference from rat leptin (<0.2%), mouse leptin (<0.2%), and human/rat insulin, proinsulin, glucagon, and IGF1 (not detectable). **Because of low reactivity of anti-human leptin antibodies, this kit is not suitable for measuring leptin from mouse/rat or other species samples.** Following cytokines also did not interfere in the assay (TNF-_, IL-2, IL-3, IL-4, IL-5, IL-6, IL-8, IL-9, IL-10, IL-12, IL-16, GM-CSF, CSF and EGF). ADI also has a mouse leptin ELISA kit (#100-130-LEM).

6. Expected Normal values

Sample	n	Leptin (ng/ml)	Range (ng/ml)
Lean Women	30	7.4	3.7-11.1
Lean Men	30	3.8	2.0-5.6

Note: As for all clinical assays, each laboratory should establish their own range of expected normal values.

6. Comparative studies

ADI's human leptin ELISA kits was compared with other commercially available competitor ELISA kit X.

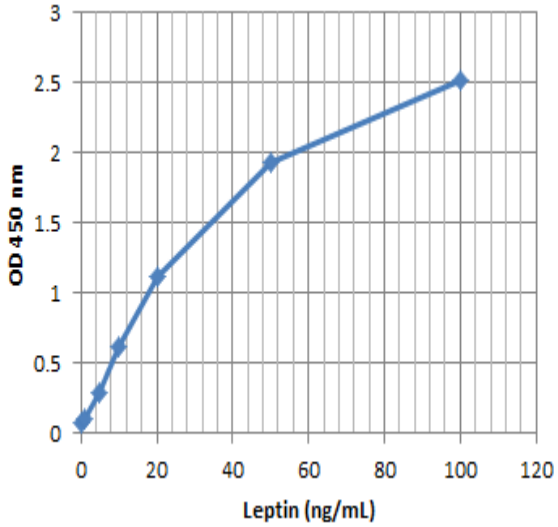
Thirty-eight serum samples ranging from 1.05-75.62 ng/mL were assayed with both kits, yielding the following results:

Regression: Kit X=0.9644 (DBC) + 1.5489 r=0.98
Kit X Mean: 21.13 ADI Mean: 20.30

WORKSHEET OF TYPICAL ASSAY

Wells	Stds/samples	Mean A_{450nm}	Calculated concn.
A1, A2	**Std. A** (0 ng/ml)	0.071	
B1, B2	**Std. B** (1 ng/ml)	0.100	
C1, C2	**Std. C** (5 ng/ml)	0.290	
D1, D2	**Std. D** (10 ng/ml)	0.625	
E1, E2	**Std. E** (20 ng/ml)	1.112	
F1, F2	**Std. F** (50 ng/ml)	1.930	
G1, G2	**Std. G** (100 ng/ml)	2.515	
S1, S2	**Sample 1**	0.275	4.22 ng/ml

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.



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

PRINCIPLE OF THE TEST

Human leptin ELISA kit is based on binding of human leptin from standards or samples to the anti-human leptin coated on the microwell plate and biotinylated antibody and subsequent detection of biotin-antibody by Streptavidin-HRP Conjugate. After a washing step, chromogenic substrate (TMB) is added and colors (blue) developed. Higher concentrations of leptin in the sample result in higher binding of antibody-enzyme (HRP) to the antibody coated plate. The enzymatic reaction (color) is directly proportional to the amount of leptin present in the sample. Adding stopping solution terminates the reaction (blue color turns yellow). Absorbance is then measured using an ELISA reader at 450 nm. and the concentration of leptin in samples and control is read off the standard curve.

MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipet (50-200 μl) and multichannel pipet with disposable plastic tips. Reagent troughs, plate shaker (orbital shaker), plate washer (recommended) and ELISA plates Reader.

PRECAUTIONS

The Alpha Diagnostic International leptin ELISA kit is intended for *in vitro research* use only. The reagents contain thimerosal or Kathon as preservative; necessary care should be taken when disposing solutions. The stds./controls sera may contain human serum that has been 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.

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.

REAGENTS PREPARATION FOR THE ASSAY

Dilute the required amount of **Streptavidin-HRP conjugate** (1:50) with the assay buffer. You will need 100 ul/well or ~10 ml for the 96 wells. Dilute as necessary.

Wash Buffer (10X): Dilute the wash buffer with distilled water (dissolve content of 1 bottle (50 ml) to 500 ml water. Some buffer components may crystallize in wash concentrate. These redissolve at room temperature. Store diluted wash buffer at 2-8oC.

STORAGE AND STABILITY

The microtiter well plate and all other reagents, if unopened, 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. Do not freeze and thaw.

TEST PROCEDURE *(ALLOW ALL REAGENTS TO REACH ROOM TEMPERATURE BEFORE USE). Prepare working solutions of Streptavidin-HRP conjugate and wash buffer. Bring all reagents and solutions to room temp. (25-28oC).*

1. Label or mark the microtiter well strips to be used on the plate.
2. Pipette **20 ul** of stds., control, and samples in duplicate into appropriate wells. Pipette **80 ul of Biotin-Anti-leptin conjugate** into each well. Mix gently for 5-10 secs.
3. **Note:** for ease of loading samples it is recommended that a second **uncoated** microwell plate should be used as a reservoir. This enables standards or samples to be transferred quickly to the ELISA plate using multichannel pipet.
4. Cover the plate and incubate for **1 hrs at room temp** (25-28oC) on a plate shaker (about 150 rpm; failure to shake will decrease kinetics). Plate can be shaken manually intermittently if a shaker is not available.
5. Aspirate and wash the wells 3 times with 300 ul 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
6. Add **100 ul of working dilution of Streptavidin-HRP** conjugate into each well. Mix gently and incubate for **30-min at room temp** (25-28oC) on a plate shaker (about 150 rpm; failure to shake will decrease kinetics) as in step 4.
7. Aspirate and wash the wells 3 times with 300 ul of diluted wash buffer as in step 5.
8. Add **100 ul of (TMB**) substrate into **each well.** Mix gently. Cover the plate and incubate for **15 minutes** (or until dark blue color develops in standard A) at room temperature on a plate shaker (about 150 rpm; failure to shake will decrease color).
9. Stop the reaction by adding 50 ul of stopping solution to **all wells** at the same timed intervals as in step 8. Mix gently.
10. Measure the absorbance at 450 nm using an ELISA reader within 15 minutes.

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 wells 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

Samples containing more 100 ng/ml leptin should be first diluted with the assay buffer or normal saline at a dilution of no more than 1:8. The results obtained should be multiplied by the appropriate first dilution factor.

CALCULATION OF RESULTS

Calculate the mean absorbance for each duplicate. Draw the standard curve on semi-log graph paper by plotting net absorbance values of standards against appropriate leptin concentrations. Read off the leptin concentrations of the control and patient samples. if using a ELISA software then we recommend point-to-point curve (do not force a linear line).

PERFORMANCE CHARACTERISTICS

1. DETECTION LIMIT

Based on sixteen replicate determinations of the zero standard, the minimum leptin concentration detectable using this assay is 0.50 ng/ml. The detection limit is defined as the value deviating by 2 SD from the zero standard.

2. PRECISION

Intra-assay precision: Two serum samples (mean leptin concentrations 4.18 and 6.81 ng/ml) were run in 10 replicates. The samples showed good intra-assay precision with %CV of 12.00 and 7.4%, respectively.

Inter-assay precision: Two serum samples were run in duplicate in 10 independent assays. The samples showed good inter-assay precision (8-9 % CV). The actual values were: mean 8.80 ng/ml, SD 0.85 ng/ml and 37.00 ng/ml, SD 3.24 ng/ml).