



Distribuito in ITALIA da
Li StarFish S.r.l.
Via Cavour, 35
20063 Cernusco S/N (MI)
telefono 02-92150794
info@listarfish.it
www.listarfish.it

Instructions for use **Leptin ELISA**

Please use only the valid version of the Instructions for Use provided with the kit

REF

ME E-0300



IVD

CE

INTENDED USE

For the quantitative determination of Leptin in human serum by an enzyme immunoassay method.

PRINCIPLE OF THE TEST

The principle of the following enzyme immunoassay test follows a typical two-step capture or 'sandwich' type assay. The assay makes use of two highly specific monoclonal antibodies: A monoclonal antibody specific for leptin is immobilized onto the microplate and another monoclonal antibody specific for a different epitope of leptin is conjugated to biotin. During the first step, leptin present in the samples and standards is bound to the immobilized antibody and to the biotinylated antibody, thus forming a sandwich complex. Excess and unbound biotinylated antibody is removed by a washing step. In the second step, streptavidin-HRP is added, which binds specifically to any bound biotinylated antibody. Again, unbound streptavidin-HRP is removed by a washing step. Next, the enzyme substrate is added (TMB), forming a blue coloured product that is directly proportional to the amount of leptin present. The enzymatic reaction is terminated by the addition of the stopping solution, converting the blue colour to a yellow colour. The absorbance is measured on a microtiterplate reader at 450 nm. A set of standards is used to plot a standard curve from which the amount of leptin in patient samples and controls can be directly read.

CLINICAL APPLICATIONS

Human Leptin is a 16 kDa, 146 amino acid residue, non-glycosylated polypeptide. Leptin is encoded by the OB gene. Its major source is the adipose tissue, and its circulating concentrations indirectly reflect body fat stores. Plasma or serum concentrations of leptin are increased in obese humans and strongly correlate with the degree of adiposity as expressed by percentage of body fat or body mass index.

The recently discovered hormone leptin contributes to the regulation of energy balance by informing the brain of the amount of adipose tissue in the body. The brain may then make the appropriate adjustments in either energy intake or expenditure.

Many areas of leptin physiology remain to be investigated. The roles of leptin in metabolism, insulin sensitivity, as a potential therapeutic modality for weight loss as well as involvement in endocrine function are active areas of research. While the future for leptin as a therapeutic agent is not clear, its involvement in many areas of physiology undoubtedly makes this a new hormone which requires extensive study in the future to understand its physiology.

PROCEDURAL CAUTIONS AND WARNINGS

1. This kit is intended for in vitro use only.
2. Practice the following good laboratory practices when handling kit reagents:
 - Do not pipette by mouth.
 - Do not smoke, drink, or eat in areas where specimens or kit reagents are being handled.
 - Wear protective clothing and disposable gloves when handling the specimens and kit reagents.
 - Wash hands thoroughly after performing the test.
 - Avoid contact with eyes; use safety glasses; in case of contact, flush with water immediately and contact a doctor.
3. Users should have a thorough understanding of this protocol for the successful use of this kit. Reliable performance will only be attained by strict and careful adherence to the instructions provided.
4. Avoid microbial contamination of reagents.
5. A standard curve must be established for every run.
6. It is recommended to all customers to prepare their own control materials or serum pools which should be included in every run at a high and low level for assessing the reliability of results.
7. The controls (included in kit) should be included in every run and fall within established confidence limits.
8. When the use of water is specified for dilution or reconstitution, use deionized or distilled water.
9. All kit reagents and specimens should be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of reagents and specimens.
10. Improper procedural techniques, imprecise pipetting, incomplete washing as well as improper reagent storage may be indicated when assay values for the control do not reflect established ranges.
11. When reading the microplate, the presence of bubbles in the wells will affect the optical densities (ODs). Carefully remove any bubbles before performing the reading step.
12. The substrate solution (TMB) is sensitive to light and should remain colourless if properly stored. Instability or contamination may be indicated by the development of a blue colour, in which case it should not be used.
13. When dispensing the substrate and stopping solution, do not use pipettes in which these liquids will come into contact with any metal parts.
14. To prevent contamination of reagents, use a new disposable pipette tip for dispensing each reagent, sample, standard and control.
15. Do not mix various lot numbers of kit components within a test and do not use any component beyond the expiration date printed on the label.

16. Kit reagents must be regarded as hazardous waste and disposed of according to local and/or national regulations.

LIMITATIONS

1. All the reagents within the kit are calibrated for the direct determination of leptin in human serum. The kit is not calibrated for the determination of leptin in saliva, plasma or other specimens of human or animal origin.
2. Do not use grossly hemolyzed, grossly lipemic, icteric or improperly stored serum.
3. Any samples or control sera containing azide or thimerosal are not compatible with this kit, as they may lead to false results.
4. Only assay buffer may be used to dilute any high serum samples. The use of any other reagent may lead to false results.
5. The results obtained with this kit should never be used as the sole basis for a clinical diagnosis. For example, the occurrence of heterophilic antibodies in patients regularly exposed to animals or animal products has the potential of causing interferences in immunological tests. Consequently, the clinical diagnosis should include all aspects of a patient's background including the frequency of exposure to animals/products if false results are suspected.

**SAFETY CAUTIONS AND WARNINGS
POTENTIAL BIOHAZARDOUS MATERIAL**

All serum samples should be considered a potential biohazard and handled with the appropriate precautions.

CHEMICAL HAZARDS

Avoid contact with reagents containing TMB, hydrogen peroxide and sulfuric acid. If contacted with any of these reagents, wash with plenty of water. TMB is a suspected carcinogen.

SPECIMEN COLLECTION AND STORAGE

Approximately 0.1 ml of serum is required per duplicate determination. Collect 4 - 5 ml of blood into an appropriately labelled tube and allow it to clot. Centrifuge and carefully remove the serum layer. Store at 4 °C for up to 24 hours or at -10 °C or lower if the analyses are to be done at a later date. Consider all human specimens as possible biohazardous materials and take appropriate precautions when handling.


REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED

1. Precision pipette to deliver 20 - 100 µl
2. Disposable pipette tips
3. Distilled or deionized water
4. Plate shaker
5. Microplate washer (recommended)
6. Microplate reader with a filter set at 450 nm and an upper OD limit of 3.0 or greater

REAGENTS PROVIDED

- 1. AA E-0030** **WASH-CONC 10x** **Wash Buffer Concentrate – Requires Preparation X10**
Contents: One bottle containing buffer with a non-ionic detergent and a non-mercury preservative.
Volume: 50 ml/bottle
Storage: Refrigerate at 2 - 8 °C
Stability: 12 months or as indicated on label
Preparation: Dilute 1:10 in distilled or deionized water before use. If the whole plate is to be used dilute 50 ml of the wash buffer concentrate in 450 ml of water.
- 2. AA E-0055** **SUBSTRATE** **TMB Substrate**
Contents: One bottle containing tetramethylbenzidine and hydrogen peroxide in a non-DMF or DMSO containing buffer.
Volume: 16 ml/bottle
Storage: Refrigerate at 2 - 8 °C
Stability: Unopened at 2 - 8 °C until expiration date on label.

3. AA E-0080 **STOP-SOLN** **Stopping Solution**

Contents: One vial containing 1M sulfuric acid.
Volume: 6 ml/bottle
Storage: Refrigerate at 2 - 8 °C
Stability: Unopened at 2 - 8 °C until expiration date on label.
Hazards identification: 

H290 May be corrosive to metals.
H314 Causes severe skin burns and eye damage.

4. Standards and Controls

Listed below are approximate concentrations, please refer to vial labels for exact concentrations:

Cat. no.	Symbol	Standard	Concentration	Volume/Vial
ME E-0301	STANDARD A	Standard A	0 ng/ml	0.5 ml
ME E-0302	STANDARD B	Standard B	1 ng/ml	0.5 ml
ME E-0303	STANDARD C	Standard C	5 ng/ml	0.5 ml
ME E-0304	STANDARD D	Standard D	10 ng/ml	0.5 ml
ME E-0305	STANDARD E	Standard E	20 ng/ml	0.5 ml
ME E-0306	STANDARD F	Standard F	50 ng/ml	0.5 ml
ME E-0307	STANDARD G	Standard G	100 ng/ml	0.5 ml
ME E-0351	CONTROL 1	Control 1	Refer to vial labels for expected value and acceptable range!	0.5 ml
ME E-0352	CONTROL 2	Control 2		0.5 ml

Contents: Leptin in a protein-based buffer with a non-mercury preservative. Prepared by spiking buffer with a defined quantity of leptin.
Storage: Refrigerate at 2 - 8 °C
Stability: Unopened at 2 - 8 °C until expiration date on label.

5. ME E-0313 **ASSAY-BUFF** **Assay Buffer**

Contents: One vial containing a protein-based buffer with a non-mercury preservative.
Volume: 20 ml/bottle
Storage: Refrigerate at 2 - 8 °C
Stability: Unopened at 2 - 8 °C until expiration date on label.

6. ME E-0331 **96** **Anti-Leptin Monoclonal Antibody-Coated Break Apart Well Microplate**

Contents: One 96 well (12x8) monoclonal antibody-coated microplate in a resealable pouch with desiccant.
Storage: Refrigerate at 2 - 8 °C
Stability: 12 months or as indicated on label.

7. ME E-0341 **BIOTIN-AB** **Monoclonal Anti-Leptin-Biotin Conjugate**

Contents: One bottle containing a monoclonal anti-leptin antibody conjugated to biotin in a protein-based buffer with a non-mercury preservative.
Volume: 10 ml/bottle
Storage: Refrigerate at 2 - 8 °C
Stability: 12 months or as indicated on label.

8. ME E-0340 **CONJUGATE-CONC 50x** **Streptavidin-HRP Conjugate Concentrate** – Requires Preparation **X50**

- Contents: One vial containing streptavidin conjugated to horseradish peroxidase in a protein-based buffer with a non-mercury preservative.
- Volume: 0.4 ml/vial
- Storage: Refrigerate at 2 - 8 °C
- Stability: Unopened at 2 - 8 °C until expiration date on label.
- Preparation: Dilute 1:50 in assay buffer before use (eg. 40 µl of concentrate in 2 ml of assay buffer). If the whole plate is to be used dilute 240 µl of concentrate in 12 ml of assay buffer. Discard any that is left over.

ASSAY PROCEDURE

All reagents must reach room temperature before use. Standards, controls and specimen samples should be assayed in duplicate. Once the procedure has been started, all steps should be completed without interruption.

1.	Prepare working solutions of the streptavidin-HRP - conjugate and wash buffer .
2.	Pipette 20 µl of each standard, control and serum samples into correspondingly labelled wells in duplicate.
3.	Pipette 80 µl of the monoclonal anti-leptin-biotin conjugate into each well.
4.	Incubate on a plate shaker (approximately 200 rpm) for 1 hour at room temperature .
5.	Wash the wells 3 times with prepared wash buffer (300 µl /well for each wash) and tap the plate firmly against absorbent paper to ensure that it is dry. (The use of a washer is highly recommended).
6.	Pipette 100 µl of prepared streptavidin-HRP - conjugate into each well.
7.	Incubate on a plate shaker (approximately 200 rpm) for 30 minutes at room temperature .
8.	Wash the wells 3 times with prepared wash buffer (300 µl /well for each wash) and tap the plate firmly against absorbent paper to ensure that it is dry. (The use of a washer is highly recommended).
9.	Pipette 100 µl of TMB substrate into each well at timed intervals.
10.	Incubate on a plate shaker for 10 - 15 minutes at room temperature .
11.	Pipette 50 µl of stopping solution into each well at the same timed intervals as in step 9.
12.	Read the plate on a micro plate reader at 450 nm within 20 minutes after addition of the stopping solution.

CALCULATIONS

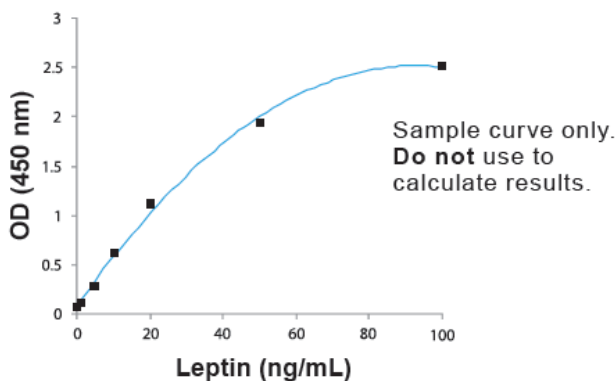
1. Calculate the mean optical density of each standard duplicate.
2. Draw a standard curve on semi-log paper with the mean optical densities on the Y-axis and the standard concentrations on the X-axis. If immunoassay software is being used, a 4-parameter or 5-parameter curve is recommended.
3. Calculate the mean optical density of each unknown duplicate.
4. Read the values of the unknowns directly off the standard curve.
5. If a sample reads more than 100 ng/ml then dilute it with assay buffer at a dilution of no more than 1:8. The result obtained should be multiplied by the dilution factor.

TYPICAL TABULATED DATA:

Sample data only. **Do not** use to calculate results.

Standard	OD 1	OD 2	Mean OD	Value (ng/ml)
A	0.073	0.070	0.072	0
B	0.102	0.100	0.101	1
C	0.290	0.293	0.292	5
D	0.620	0.630	0.625	10
E	1.140	1.086	1.113	20
F	1.947	1.919	1.933	50
G	2.518	2.514	2.516	100
Unknown	0.275	0.273	0.274	4.22

TYPICAL STANDARD CURVE



PERFORMANCE CHARACTERISTICS

SENSITIVITY

The limit of detection (LoD) for Leptin is 0.50 ng/ml, as determined by use of a NCCLS protocol and with proportions of false positives (α) less than 5 % and false negatives (β) less than 5 %; based on 82 blank determinations; LoB=0.42 ng/ml.

SPECIFICITY (CROSS-REACTIVITY)

The following substances were tested at 1000 ng/ml and exhibited no cross-reactivity: Mouse Leptin, TNF- α , 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.

INTRA-ASSAY PRECISION

Four serum samples were assayed twenty times each on the same standard curve. The results (in ng/ml) are tabulated below:

Sample	Mean	SD	CV%
1	2.45	0.09	3.7
2	7.94	0.34	4.3
3	11.67	0.64	5.5
4	27.51	1.37	5.0

INTER-ASSAY PRECISION

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

Sample	Mean	SD	CV%
1	2.71	0.16	5.9
2	8.24	0.48	5.8
3	12.01	0.82	6.8
4	24.98	1.45	5.8

RECOVERY

Spiked samples were prepared by adding defined amounts of leptin to three patient serum samples. The results (in ng/ml) are tabulated below:

Sample	Observed	Expected	% Recovery
1 Unspiked	3.89	-	-
+ 3.06	6.28	6.95	90.4
+ 8.06	10.98	11.95	91.9
+ 23.06	25.43	26.95	94.4
2 Unspiked	7.89	-	-
+ 1.06	8.82	8.95	98.5
+ 6.06	15.03	13.95	107.7
+ 21.06	30.32	28.95	104.7
3 Unspiked	11.61	-	-
+ 4.2	15.71	15.81	99.4
+ 12.8	25.42	24.41	104.1
+ 29.46	41.18	41.07	100.3

LINEARITY

Three patient serum samples were serially diluted with leptin assay buffer. The results (in ng/ml) are tabulated below:

Sample	Observed	Expected	% Recovery
1	3.03	-	-
1:2	1.42	1.52	93.4
1:4	0.71	0.76	93.4
1:8	0.35	0.38	92.1
2	11.27	-	-
1:2	5.93	5.64	105.1
1:4	3.05	2.82	108.2
1:8	1.35	1.41	95.7
3	27.91	-	-
1:2	14.91	13.96	106.8
1:4	6.74	6.98	96.6
1:8	3.29	3.49	94.3

COMPARATIVE STUDY

The Leptin ELISA was compared against a leading competitor's Leptin EIA kit (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 (Leptin ELISA) + 1.5489

r = 0.98

Kit X Mean: 21.13

Leptin ELISA Mean: 20.30

EXPECTED NORMAL VALUES

As for all clinical assays, each laboratory should collect data and establish their own range of expected normal values.

Group	Mean (ng/ml)	Range (ng/ml)
Lean Women	7.4	3.7 - 11.1
Lean Men	3.8	2.0 - 5.6







Leptin values are approximately 2.5 times higher in women than men per unit BMI.

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8. Tamura T, et al. Serum Leptin Concentrations During Pregnancy and Their Relationship to Fetal Growth. *Obstet Gynecol*. 1998; 91(3):389–95.
9. Matsuda J, et al. Serum Leptin Concentration in Cord Blood: Relationship to Birth Weight and Gender. *J Clin Endocrinol Metab*. 1997; 82(5):1642–4.
10. Check JH, et al. Falsely Elevated Steroidal Assay Levels Related to Heterophile Antibodies Against Various Animal Species. *Gynecol Obstet Invest*. 1995; 40(2): 139–40.

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Symbols:

	Storage temperature		Manufacturer		Contains sufficient for <n> tests
	Expiry date	LOT	Batch code	IVD	For in-vitro diagnostic use only!
	Consult instructions for use	CONT	Content	CE	CE labelled
	Caution	REF	Catalogue number		