



# Leptin ELISA Assay Kit

Catalog Number:

**LPT31-K01 (1 x 96 wells)**

For Research Use Only. Not for use in diagnostic procedures.

v. 11.0

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**Eagle Biosciences, Inc.**

20A Northwest Blvd., Suite 112, Nashua, NH 03063

Phone: 617-419-2019 Fax: 617-419-1110

**[www.EagleBio.com](http://www.EagleBio.com)**



## **INTENDED USE**

The Eagle Biosciences Leptin ELISA Assay Kit (enzyme-linked immunoassay kit) is intended for the quantitative determination of leptin in human serum. The Eagle Biosciences Leptin ELISA Assay Kit is for research use only and not to be used in diagnostic procedures.

## **INTRODUCTION**

Human Leptin is a 16 kDa, 146 amino acid residue, nonglycosylated 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.

## **PRINCIPLE OF THE ASSAY**

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 colored 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 color to a yellow color. The absorbance is measured on a microtiter plate 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.

## **PROCEDURAL CAUTIONS AND WARNINGS**

1. This kit is intended for research 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 calibrator 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 colorless if properly stored. Instability or contamination may be indicated by the development of a blue color, 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. This kit is intended for research use only and should not be used as a diagnostic tool.

#### **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 pipettes to dispense 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\* (see assay procedure step 10)

## REAGENTS PROVIDED

### 1. 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.

### 2. 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.

### 3. 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.



#### 4. Leptin Calibrators

Contents: Seven vials containing leptin in a protein-based buffer with a nonmercury preservative. Prepared by spiking buffer with a defined quality of leptin.

\*Listed below are approximate concentrations, please refer to vial labels for exact concentrations.

Calibrator	Concentration	Volume/Vial
A	0 ng/mL	0.5 mL
B	1 ng/mL	0.5 mL
C	5 ng/mL	0.5 mL
D	10 ng/mL	0.5 mL
E	20 ng/mL	0.5 mL
F	50 ng/mL	0.5 mL
G	100 ng/mL	0.5 mL

Storage: Refrigerate at 2–8°C.

Stability: Unopened at 2–8°C until expiration date on label.

#### 5. Controls

Contents: Two vials containing leptin in a protein-based buffer with a nonmercury preservative. Prepared by spiking buffer with defined quantities of leptin. Refer to bottle labels for the acceptable range.

Volume: 0.5 mL/vial

Storage: Refrigerate at 2–8°C

Stability: Unopened at 2–8°C until expiration date on label.

#### 6. 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 one whole plate is to be used dilute 50 mL of the wash buffer concentrate in 450 mL of water.

#### 7. Assay Buffer

Contents: One bottle 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.



## 8. 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.

## 9. Stopping Solution — Ready To Use

Contents:	One bottle 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.

## ASSAY PROCEDURE

All reagents must reach room temperature before use. Calibrators, 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  $\mu$ L of each calibrator, control and specimen sample into correspondingly labelled wells in duplicate.
3. Pipette 80  $\mu$ L 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  $\mu$ L/well for each wash) and tap the plate firmly against absorbent paper to ensure that it is dry. (The use of a washer is recommended.)
6. Pipette 100  $\mu$ L 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 again in the same manner as step 5.
9. Pipette 100  $\mu$ L 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  $\mu$ L of stopping solution into each well at the same timed intervals as in step 9.
12. Read the plate on a microplate reader at 450 nm within 20 minutes after addition of the stopping solution.

## CALCULATIONS

1. Calculate the mean optical density of each calibrator duplicate.
2. Draw a calibrator curve on semi-log paper with the mean optical densities on the Y-axis and the calibrator 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 calibrator 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.

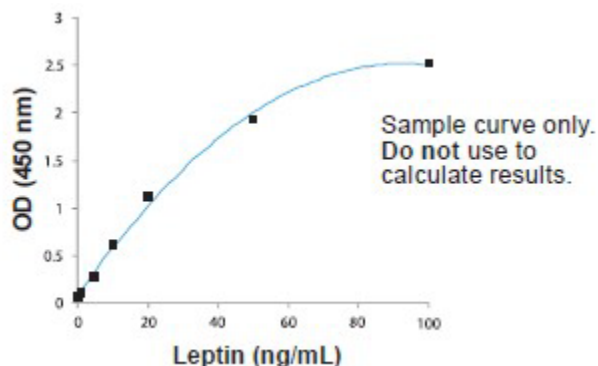


### TYPICAL TABULATED DATA

Sample data only. Do not use to calculate results.

Calibrator	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 CALIBRATOR 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 ( $\alpha$ ) less than 5% and false negatives ( $\beta$ ) 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- $\alpha$ , 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 calibrator 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	Obs. Result	Exp. Result	Recovery %
1 Unspiked	3.89	-	-
+ 3.06	6.28	6.95	90.4
+ 8.06	10.98	11.95	91.9
+ 23.00	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
+ 29.5	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	Obs. Result	Exp. Result	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 (This Kit) + 1.5489

r = 0.98

Kit X Mean: 21.13

Mean (This kit): 20.30

### EXPECTED 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.

### REFERENCES

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## **Warranty Information**

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For further information about this kit, its application or the procedures in this kit, please contact the Technical Service Team at Eagle Biosciences, Inc. at [info@eaglebio.com](mailto:info@eaglebio.com) or at 866-411-8023.