Product Manual

Human Leptin ELISA Kit

Catalog Numbers

MET-5057	96 assays
MET-5057-5	5 x 96 assays

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

Introduction

Leptin is a polypeptide hormone responsible for regulating adipose tissue mass and energy levels by inhibiting hunger. Also known as the "satiety hormone," leptin counteracts the "hunger hormone" ghrelin, which regulates appetite and the distribution and rate of energy use. Leptin is the product of the Ob gene, which is secreted by adipocytes as a 16 kDa protein, or bound to soluble form of its receptor (Ob-R). The hormone principally acts on the hypothalamus and hippocampus to decrease food intake and adjust fat and glucose metabolism. Leptin levels are positively related to body fat mass with increased levels leading to a negative energy balance and while decreased levels lead to a positive balance. There is a decreased sensitivity to leptin in obese conditions which results in an inability to detect satiety in spite of high energy reserves. It is implicated in many endocrine functions ranging from adiposity, energy homeostasis, satiety, and fertility and puberty. Pathways involving T cells activity, pancreatic islets, inflammatory responses, bone mass and metabolism, Alzheimer's disease, vascular chemistry, and thyroid hormone production are all known to be influenced by leptin. Therefore, leptin is an excellent biological marker for understanding a variety of disease states and disorders.

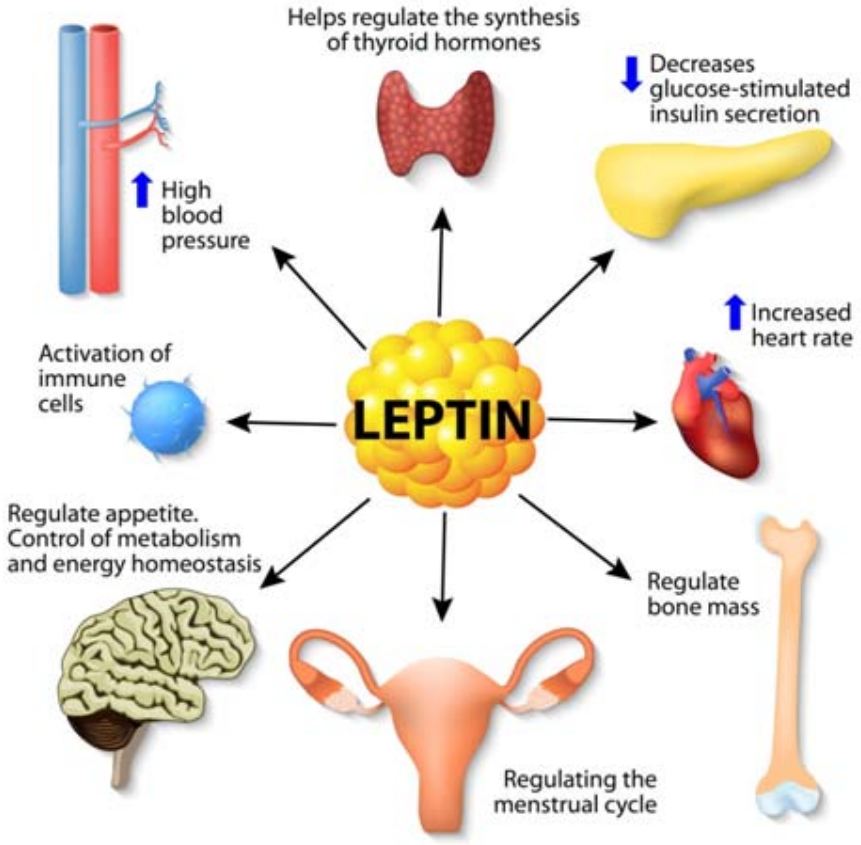

Figure 1: Leptin's Diverse Pathways

Cell Biolabs' Human Leptin ELISA Kit is an enzyme immunoassay developed for detection and quantitation of human leptin. The kit utilizes a recombinant human leptin standard and has a detection sensitivity limit of 2 pg/mL. Each kit provides sufficient reagents to perform up to 96 assays including the standard curve and samples.

Assay Principle

This assay is based on a sandwich ELISA format. Leptin present in samples or standards bind to the anti-leptin antibodies pre-adsorbed on the microtiter plate. Next, a biotinylated anti-leptin antibody is added to the plate well and binds to the captured leptin. A streptavidin-enzyme conjugate is then added, which binds to the biotin of the second antibody. Unbound streptavidin-enzyme conjugate is removed during a wash step, and substrate solution is added to the wells. A colored product is formed in proportion to the amount of leptin present in the sample. The reaction is terminated by addition of acid and absorbance is measured at 450 nm. A standard curve is prepared from purified recombinant human leptin. Sample concentration is then determined by comparing to the known values of the standard curve.

Related Products

1. CBA-290: CytoSelect™ 96-Well Adipogenesis Assay Kit
2. MET-5051: Human Thyroid-Stimulating Hormone (TSH) ELISA Kit
3. MET-5052: Human Adiponectin ELISA Kit
4. STA-384: Total Cholesterol Assay Kit (Colorimetric)
5. STA-390: Total Cholesterol Assay Kit (Fluorometric)
6. STA-396: Serum Triglyceride Quantitation Kit (Colorimetric)
7. STA-618: Free Fatty Acid Assay Kit (Colorimetric)
8. STA-680: Glucose Assay Kit (Colorimetric)

Kit Components

Box 1 (shipped at room temperature)

1. Anti-Leptin Antibody Coated Plate (Part No. 50571B): One strip well 96-well plate
2. Anti-Leptin Biotinylated Antibody (1000X) (Part No. 50572D): One 10 µL vial of anti-leptin antibody
3. Streptavidin-Enzyme Conjugate (Part No. 310803): One 20 µL tube
4. Assay Diluent (Part No. 310804): One 50 mL bottle
5. 10X Wash Buffer (Part No. 310806): One 100 mL bottle
6. Substrate Solution (Part No. 310807): One 12 mL amber bottle
7. Stop Solution (Part. No. 310808): One 12 mL bottle

Box 2 (shipped on blue ice packs)

1. Leptin Standard (Part No. 50573D): One 10 µL vial of 10 µg/mL human leptin

Materials Not Supplied

1. Leptin samples: human serum, plasma, lysates
2. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
3. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
4. Multichannel micropipette reservoir
5. Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)

Storage

Upon receipt, aliquot and store Leptin Standard at -80ºC and avoid freeze/thaw. Store all other components at 4ºC.

Preparation of Reagents

- 1X Wash Buffer: Dilute the 10X Wash Buffer to 1X with deionized water. Stir to homogeneity.
- Biotinylated Anti-Leptin Antibody and Streptavidin-Enzyme Conjugate: Immediately before use dilute the Biotinylated Anti-Leptin Antibody 1:1000 and the Streptavidin-Enzyme Conjugate 1:1000 with Assay Diluent. Do not store diluted solutions.
- Substrate Solution: Prior to use, warm the Substrate Solution to room temperature.

Preparation of Samples

Samples should be assayed immediately or stored at -80ºC prior to performing the assay. Optimal experimental conditions for samples must be determined by the investigator. The following recommendations are only guidelines and may be altered to optimize or complement the user's experimental design. A set of serial dilutions is recommended for samples to achieve optimal assay results and minimize possible interfering compounds. Run proper controls as necessary. Always run a standard curve with samples.

- Plasma: Collect blood with an anticoagulant such as heparin, citrate or EDTA and mix by inversion. Centrifuge the blood at 1000 x g at 4°C for 10 minutes. Remove the plasma and assay immediately or store samples at -80ºC for up to three months. Normal plasma samples require about 10 to 1000 fold dilution with 1X PBS containing 0.1% BSA immediately before running the ELISA.
- Serum: Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 2500 x g for 20 minutes. Remove the yellow serum supernatant without disturbing the white buffy layer. Assay immediately or store samples at -80ºC for up to three months. Normal serum samples require about 10 to 1000 fold dilution with 1X PBS containing 0.1% BSA immediately before running the ELISA.
- Cell or Tissue Lysate: Sonicate or homogenize sample in cold PBS and centrifuge at 10,000 x g for 10 minutes at 4ºC. Assay immediately or store samples at -80ºC for up to three months. Dilute samples in 1X PBS containing 0.1% BSA as needed.

- Other Biological Fluids: Centrifuge samples for 10 minutes at 1000 g at 4ºC. Assay immediately or store samples at -80ºC for up to three months. Dilute samples in 1X PBS containing 0.1% BSA as needed.

Preparation of Standard Curve

1. Prepare fresh standards by diluting the Leptin Standard stock tube from 10 µg/mL to 10 ng/mL (1:1000) in Assay Diluent (Example: Add 2 µL of Leptin Standard stock tube to 1.998 mL of Assay Diluent).
2. Prepare a series of the remaining leptin standards in the concentration range of 250 pg/mL – 2 pg/mL by diluting the 10 ng/mL according to Table 1 below.

Standard Tubes	10 ng/mL Human Leptin Standard (µL)	Assay Diluent (µL)	Leptin (pg/mL)
1	25	975	250
2	500 of Tube #1	500	125
3	500 of Tube #2	500	62.5
4	500 of Tube #3	500	31.3
5	500 of Tube #4	500	15.6
6	500 of Tube #5	500	7.8
7	500 of Tube #6	500	3.9
8	500 of Tube #7	500	2.0
9	0	500	0

Table 1. Preparation of Leptin Standard Curve.

Note: Do not store diluted leptin standard solutions.

Assay Protocol

Note: Each leptin standard and unknown samples should be assayed in duplicate or triplicate. A freshly prepared standard curve should be used each time the assay is performed.

1. Add 100 µL of leptin standards or samples to the Anti-Leptin Antibody Coated Plate. Each sample, standard, blank, and control should be assayed in duplicate.
2. Incubate 1 hour at room temperature on an orbital shaker.
3. Remove the solution from the wells. Wash microwell strips 5 times with 250 µL 1X Wash Buffer per well with thorough aspiration between each wash. After the last wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess 1X Wash Buffer.
4. Add 100 µL of the diluted Biotinylated Anti-Leptin Antibody to each well.
5. Incubate 1 hour at room temperature on an orbital shaker.
6. Remove the solution from the wells. Wash the strip wells 5 times according to step 3 above.

7. Add 100 µL of the diluted Streptavidin-Enzyme Conjugate to each well.
8. Incubate 1 hour at room temperature on an orbital shaker.
9. Remove the solution from the wells. Wash the strip wells 5 times according to step 3 above.
10. Add 100 µL of Substrate Solution to each well, including the blank wells. Incubate at room temperature on an orbital shaker. Actual incubation time may vary from 5-20 minutes.

    Note: Watch plate carefully; if color changes rapidly, the reaction may need to be stopped sooner to prevent saturation.
11. Stop the enzyme reaction by adding 100 µL of Stop Solution into each well, including the blank wells. Results should be read immediately (color will fade over time).
12. Read absorbance of each microwell on a spectrophotometer using 450 nm as the primary wave length.

Example of Results

The following figures demonstrate typical Human Leptin ELISA results. One should use the data below for reference only. This data should not be used to interpret actual results.

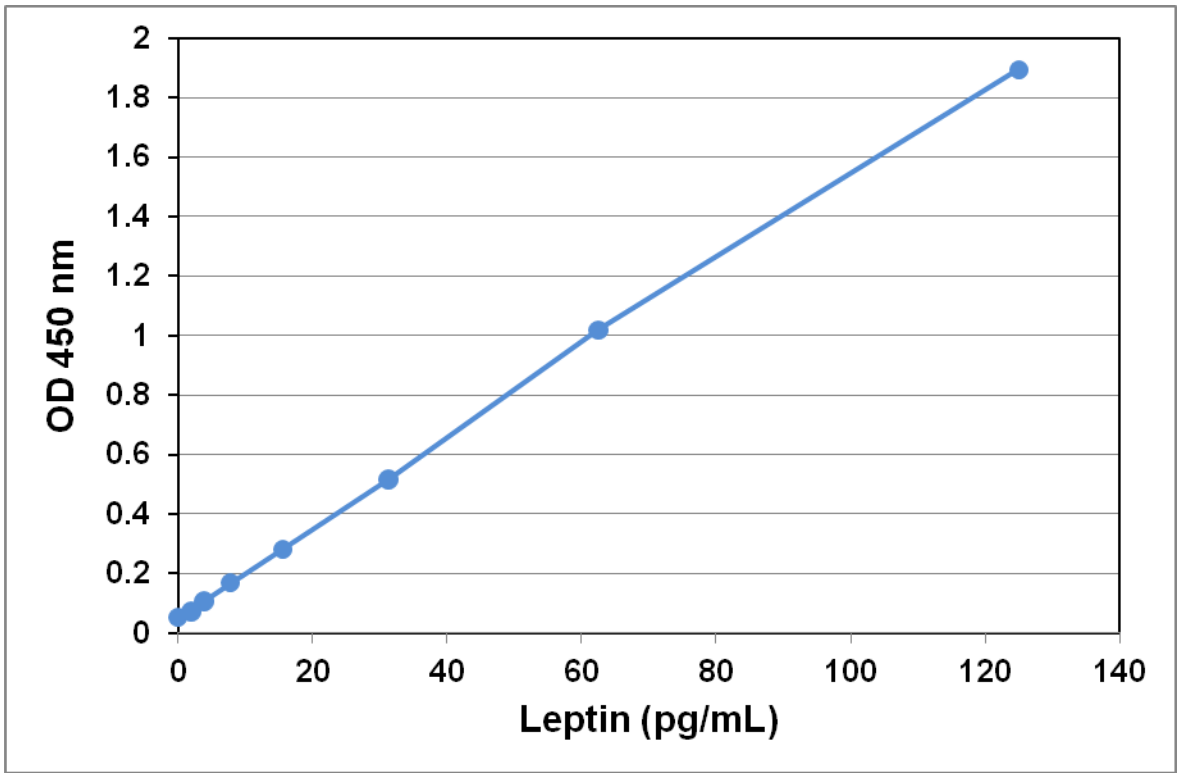

Figure 2: Human Leptin ELISA Standard Curve

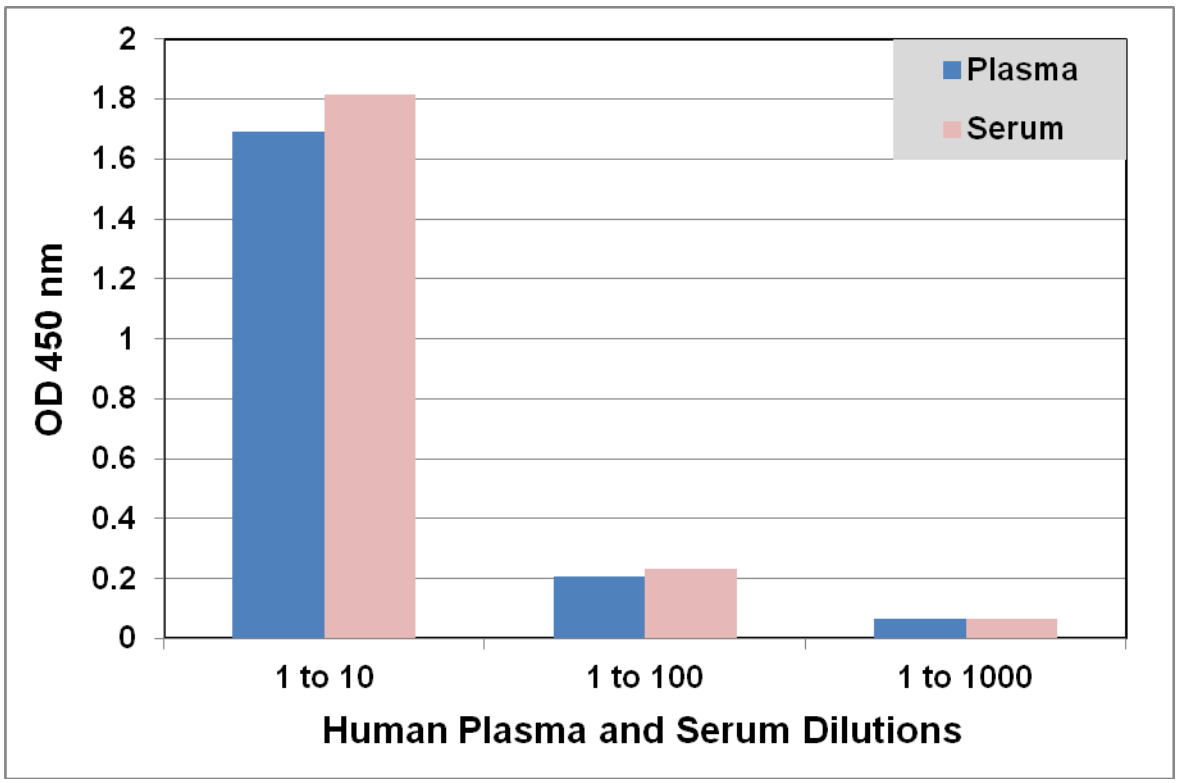

Figure 3: Leptin Levels in Human Plasma and Serum. Human Plasma and Serum were diluted 1:10, 1:100 and 1:1000 in Assay Diluent and tested according to the product insert.

References

1. Brennan A.M., et al. (2006) *Endocrinol. Metab.* **2(6)**: 318-327.
2. Frieman, J., et al. (1998) *Nature* **395**: 763-769.
3. Tartaglia, L.A., et al. (1995) *Cell* **83(7)**: 1263-1271.
4. Zhang, Y., et al. (2006) *Physiol. Behav.* **88**: 249-256.

Warranty

Contact Information

Cell Biolabs, Inc.
7758 Arjons Drive
San Diego, CA 92126
Worldwide: +1 858-271-6500
USA Toll-Free: 1-888-CBL-0505
E-mail: tech@cellbiolabs.com
www.cellbiolabs.com