BioLegend®

Enabling Legendary Discovery™

LEGEND MAX™

ELISA Kit

Human Leptin

Cat. No. 444307

ELISA Kit for Accurate Quantitation of
Human Leptin
in Serum, Plasma, and Cell Culture Supernatant

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biolegend.com

It is highly recommended that this manual be read in its entirety before using this product. Do not use this kit beyond the expiration date.

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LEGEND MAX™ Human Leptin ELISA Kit

Introduction:

Human Leptin is a 167 amino acid protein and approximately 16 kDa in mass. Leptin is encoded by the obese (ob) gene and the gene locates on chromosome 7. Leptin is an adipocyte-derived hormone and mainly secreted by adipocyte; small amounts of Leptin are expressed by the cells in the epithelium of the stomach and in the placenta. Leptin's effects on body weight are mediated through impacts on hypothalamic centers that control feeding behavior, hunger, body temperature, and energy expenditure. Leptin importantly regulates food intake, body weight, metabolism, energy expenditure, reproductive function, and adiposity through hypothalamic Leptin receptor. Leptin is also the member of long-chain helical cytokine family.

The LEGEND MAX™ Human Leptin ELISA Kit is a Sandwich Enzyme-Linked Immunosorbent Assay (ELISA) with a 96-well strip plate that is pre-coated with anti-human Leptin antibody. The detection antibody is a biotinylated anti-human Leptin antibody. This kit is specifically designed for the accurate quantitation of human Leptin in human serum, plasma, and cell culture supernatant. It is analytically validated with ready-to-use reagents.

Materials Provided:

Description	Quantity	Volume	Part #
Human Leptin pre-coated 96-well Strip Microplate	1 plate		750003960
Human Leptin Detection Antibody	1 bottle	12 mL	750003962
Human Leptin Lyophilized Standard	1 vial	Lyophilized	750003713
Avidin HRP	1 bottle	12 mL	77897
Assay Buffer B	1 bottle	25 mL	79128
Wash Buffer (20x)	1 bottle	50 mL	78233
Substrate Solution F	1 bottle	12 mL	79132
Stop Solution	1 bottle	12 mL	79133
Plate Sealers	4 sheets		78101

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Materials to be Provided by the End-User:

- Microplate reader able to measure absorbance at 450 nm
- Adjustable pipettes to measure volumes ranging from 1 µL to 1,000 µL
- Deionized water
- Wash bottle or automated microplate washer
- Log-Log graph paper or software for data analysis
- Polypropylene tubes to prepare standard dilutions
- Timer
- Plate Shaker
- Polypropylene vials

Storage Information:

Store unopened kit components between 2°C and 8°C. Do not use this kit beyond its expiration date.

Opened or Reconstituted Components	
Microplate wells	If not all microplate strips are used, remove the excess strips by pressing up from underneath each strip. Place excess strips back in the foil pouch with the included desiccant pack and reseal. Store between 2°C and 8°C for up to one month.
Standard	The remaining reconstituted standard stock solution can be aliquoted into polypropylene vials and stored at -70°C for up to one month. Avoid repeated freeze-thaw cycles.
Avidin-HRP	Store opened reagent bottles at 2° - 8°C and use within 1 month
Assay Buffer B	
Wash Buffer (20X)	
Substrate Solution F	
Stop Solution	

Health Hazard Warnings:

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin. Refer to the MSDS online at BioLegend's website for details (www.biolegend.com/msds).
2. Substrate Solution F is harmful if inhaled or ingested. Avoid skin, eye and clothing contact.
3. To reduce the likelihood of blood-borne transmission of infectious agents,

handle all serum, plasma and other biological fluids in accordance with NCCLS regulations.

4. Stop Solution contains strong acid. *Wear eye, hand, and face protection.*
5. Before disposing of the plate, rinse it with an excess amount of tap water.

Specimen Collection and Handling:

Specimens should be clear and non-hemolyzed. If possible, unknown samples should be run at a number of dilutions to determine the optimal dilution factor that will ensure accurate quantitation.

Cell Culture Supernatant: If necessary, centrifuge all samples to remove debris prior to analysis. It is recommended that samples be stored at < -70°C. Avoid repeated freeze-thaw cycles.

Serum: Use a serum separator tube and allow clotting for at least 30 minutes, then centrifuge for 10 minutes at 1,000 x *g*. Remove serum layer and assay immediately or store serum samples at < -70°C. Avoid repeated freeze-thaw cycles.

Plasma: Collect blood samples in citrate, heparin or EDTA containing tubes. Centrifuge for 10 minutes at 1,000 x *g* within 30 minutes of collection. Assay immediately or store plasma samples at < -70°C. Avoid repeated freeze-thaw cycles.

Reagent and Sample Preparation:

Note: All reagents should be diluted immediately prior to use.

1. Dilute the 20X Wash Buffer to 1X with deionized water. For example, make 1 liter of 1X Wash Buffer by adding 50 mL of 20X Wash Buffer to 950 mL of deionized water. If crystals have formed in the 20X Wash Buffer, bring to room temperature and vortex until dissolved.
2. Reconstitute the lyophilized Human Leptin Lyophilized Standard by adding the volume of Assay Buffer B to make the 30 ng/mL standard stock solution (Refer to LEGEND MAX Kit Lot-Specific Certificate of Analysis/LEGEND MAX Kit Protocol). Allow the reconstituted standard to sit at room temperature for 15-20 minutes, then briefly vortex to mix completely.
3. In general, 10-folds dilution in Assay Buffer B is recommended for serum and plasma samples. Cell culture supernatant should be tested initially without any dilution. Samples can be diluted further in Assay Buffer B to fit within the range of the assay as determined by the end user.

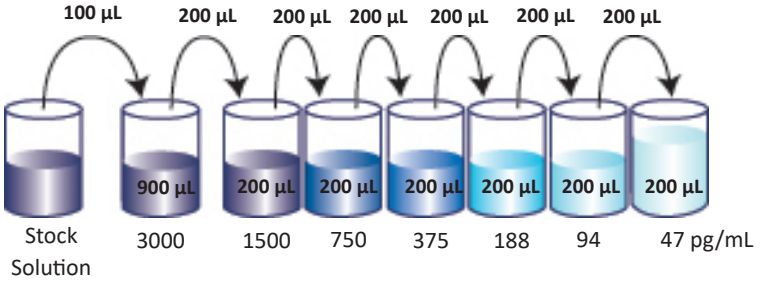
Assay Procedure:

Note: Do not mix reagents from different kits or lots. Reagents and/or antibodies from different manufacturers should not be used with this kit.

1. Bring all reagents to room temperature prior to use. It is strongly recommended that all standards and samples be run in duplicate or triplicate. A standard curve is required for each assay.

2. If not all microplate strips will be used, remove the excess strips by pressing up from underneath each strip. Place excess strips back in the foil pouch with the included desiccant pack and reseal.

3. Prepare 1000 µL of the 3000 pg/mL top standard by adding 100 uL of the 30 ng/mL standard stock solution into 900 µL Assay Buffer B. Perform six two-fold serial dilutions of the 3000 pg/mL top standard in separate tubes using Assay Buffer B as the diluent. Thus, the human Leptin standard concentrations in the tubes are 3000 pg/mL, 1500 pg/mL, 750 pg/mL, 375 pg/mL, 188 pg/mL, 94 pg/mL and 47 pg/mL, respectively. Assay Buffer B serves as the zero standard (0 pg/mL).



4. Wash the plate 4 times with at least 300 µL of 1X Wash Buffer per well and blot any residual buffer by firmly tapping the plate upside down on absorbent paper. All subsequent washes should be performed similarly.

5. Add 50 µL of Assay Buffer B to each well that will contain either standard dilutions or samples. Then add 50 µL of standard dilutions or samples to the appropriate wells.

6. Seal the plate with a Plate Sealer included in the kit and incubate the plate for 2 hours at room temperature with shaking.

7. Discard the contents of the plate into a sink, then wash the plate 4 times with 1X Wash Buffer as in step 4.

8. Add 100 µL of Human Leptin Detection Antibody solution to each well, seal the plate and incubate at room temperature for 1 hour while shaking.

9. Discard the contents of the plate into a sink, then wash the plate 4 times with 1X Wash Buffer as in step 4.

10. Add 100 µL of Avidin-HRP solution to each well, seal the plate and incubate at room temperature for 30 minutes while shaking.

11. Discard the contents of the plate into a sink, then wash the plate 5 times with 1X Wash Buffer as in step 4. For this final wash, soak wells in 1X Wash Buffer for 30 seconds to 1 minute for each wash. This will help minimize background.

12. Add 100 µL of Substrate Solution F to each well and incubate for 10 minutes in the dark. Wells containing Human Leptin should turn blue in color with an intensity proportional to its concentration. It is not necessary to seal the plate during this step.

13. Stop the reaction by adding 100 µL of Stop Solution to each well. The solution color should change from blue to yellow.

14. Read absorbance at 450 nm within 30 minutes. If the reader is capable of reading at 570 nm, the absorbance at 570 nm can be subtracted from the absorbance at 450

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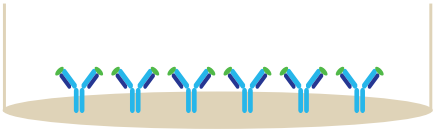
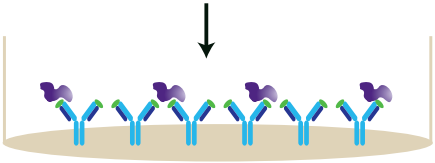
Assay Procedure Summary

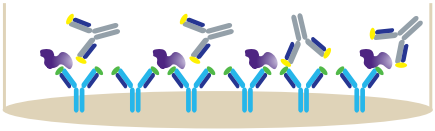
1. Wash 4 times.
   Add 50 µL Assay Buffer B to standard wells and sample wells



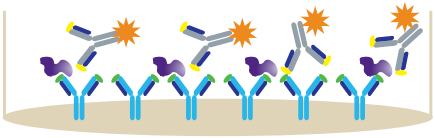
2. Add 50 µL of standard or sample, incubate 2 hr, RT, shaking



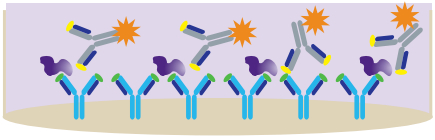
3. Wash 4 times
   Add 100 µL of Human Leptin Detection Antibody solution.
   Incubate 1 hr, RT, shaking



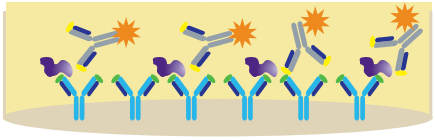
4. Wash 4 times
   Add 100 µL Avidin-HRP solution.
   Incubate 30 min, RT, shaking



5. Wash 5 times
   Add 100 µL Substrate Solution F
   Incubate 10 min, RT, in the dark



6. Add 100 µL Stop Solution



7. Read absorbance at 450 nm and 570 nm

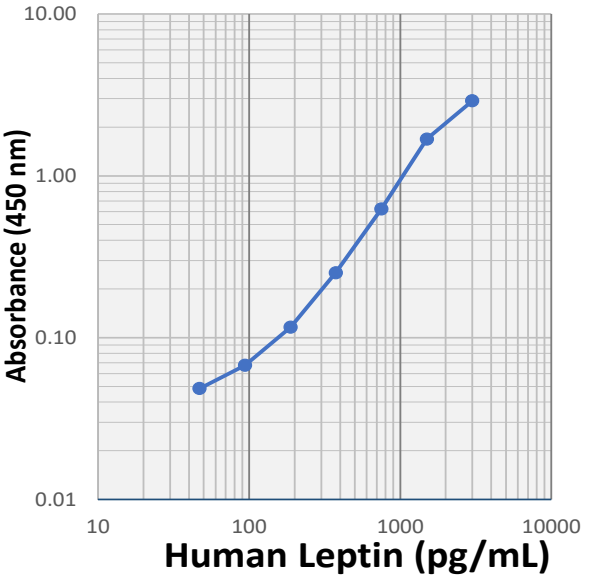
Calculation of Results:

The data can be best calculated with computer-based curve-fitting software using a 5- or 4-parameter logistics curve-fitting algorithm. If an appropriate software is not available, use log-log graph paper to determine sample concentrations. Determine the mean absorbance for each set of duplicate or triplicate standards, controls, and samples. Plot the standard curve on log-log graph paper with analyte concentration on the X-axis and absorbance on the Y-axis. Draw a best fit line through the standard points. To determine the unknown analyte concentrations, find the mean absorbance value of the unknown concentration on the Y-axis and draw a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the analyte concentration.

If samples were diluted, multiply the concentration by the appropriate dilution factor. If a test sample's absorbance value falls outside the linear portion of the standard curve, the test sample needs to be re-analyzed at a higher (or lower) dilution as appropriate.

Typical Data:

This standard curve was generated at BioLegend for demonstration purposes only. A standard curve must be run with each assay.



Performance Characteristics:

Specificity: This kit recognizes natural and recombinant Human Leptin. No cross reactivity was observed when this kit was used to analyze the following recombinant proteins at 20 ng/mL.

Human	IL-6, IL-11, IL-12, and Oncostatin M (OSM)

Sensitivity: The minimum detectable concentration of Human Leptin is 31.7± 12.2 pg/mL (n=6).

Recovery: Recombinant Human Leptin at 3 different concentrations was spiked into human samples: Serum, Citrate Plasma, EDTA Plasma, and Heparin Plasma, and cell culture supernatant. Sample recovery was then analyzed with the LEGEND MAX™ Human Leptin Kit.

Sample Type	N	% Recovery
Serum	3	92%
Citrate Plasma	3	87%
EDTA Plasma	3	100%
Heparin Plasma	3	94%
Cell Culture Supernatant	1	106%

Linearity: Natural human Serum and Plasma samples were first diluted 4 folds. No initial dilution was done to human cell culture supernatant. Then, the samples were diluted 2 fold in serial to produce samples within the dynamic range of the kit. Samples were then assayed to determine the dilutional linearity.

Sample Type	N	% Linearity
Serum	3	106%
Citrate Plasma	3	103%
EDTA Plasma	3	104%
Heparin Plasma	3	104%
Cell Culture Supernatant	1	96%

Intra-Assay Precision: Two samples containing different human Leptin concentrations were tested on one plate with 16 replicates.

Concentration	Sample 1A	Sample 2A
Number of Replicates	16	16
Mean Concentration (pg/mL)	972	203
Standard Deviation	27.4	5.9
%CV	2.8%	2.9%

Inter-Assay Precision: Two samples containing different human Leptin concentrations were tested in ten independent assays.

Concentration	Sample 1	Sample 2
Number of Assays	10	10
Mean Concentration (pg/mL)	1140	250
Standard Deviation	73.7	23.2
%CV	6.5%	9.3%

Biological Samples: Human Serum, Citrate Plasma, EDTA Plasma, and Heparin Plasma were assayed for natural human leptin.

	Serum	Citrate Plasma	EDTA Plasma	Heparin Plasma
N	9	6	5	6
Min (ng/mL)	0.3	2.1	2.2	1.0
Max (ng/mL)	28.0	19.4	20.5	18.5
Mean (ng/mL)	7.2	7.0	9.1	8.0

The pre-adipocytes are isolated from human subcutaneous adipose tissue. The adipocytes are differentiated in vitro using these isolated pre-adipocytes. The isolated pre-adipocytes are plated into the culture ware and treated with Adipocyte Differentiation Medium (DM-2) to stimulate adipocyte differentiation. Oil droplets should appear within 4-7 days after differentiation is induced. Mature adipocytes (greater than 2 weeks post-differentiation) secrete Leptin from cells in 24 hours. Human subcutaneous pre-adipocytes and adipocytes were assayed for Human Leptin.

	Pre-adipocytes	Adipocytes
pg/mL	ND	286

Troubleshooting Guide:

Problem	Probable Cause	Solution
High Background	Background wells were contaminated	Avoid cross-well contamination by using the provided plate sealers. Use multichannel pipettes and change tips between pipetting samples and reagents.
	Insufficient washes	Increase number of washes. Increase soaking time between washes prior to addition of substrate solution.
	TMB Substrate Solution was contaminated	TMB Substrate Solution should be clear and colorless prior to addition to wells. Use a clean container prior to pipetting substrate solution into wells.
No or poor signal	Detection Antibody, Avidin-HRP or Substrate solution were NOT added	Rerun the assay and follow the protocol.
	Wrong reagent or reagents were added in wrong sequential order	
	Insufficient plate agitation	The plate should be agitated during all incubation steps using a plate shaker at a speed where solutions in wells are within constant motion without splashing.
	The wash buffer contains Sodium Azide (NaN3)	Avoid Sodium Azide contamination in the wash buffer as it inhibits HRP activity.
	Incubations were done at an inappropriate temperature, timing or without agitation	Rerun the assay and follow the protocol.
Low or poor standard curve signal	The standard was incorrectly reconstituted or diluted	Adjust the calculations and follow the protocol.
	Standard was inappropriately stored	Store the reconstituted standard stock solution in polypropylene vials at -70°C. Avoid repeated freeze-thaw cycles.
	Reagents added to wells with incorrect concentrations	Check for pipetting errors and the correct reagent volume.

Problem	Probable Cause	Solution
Signal is high, standard curves have saturated signal	Standard reconstituted with less volume than required	Reconstitute new lyophilized standard with the correct volume of solution recommended in the protocol.
	Standards/samples, detection antibody, Avidin-HRP or substrate solution were incubated for too long	Rerun the assay and follow the protocol.
Sample readings are out of range	Samples contain no or below detectable levels of the analyte	If samples are below detectable levels, it may be possible to use a larger sample volume. Contact technical support for appropriate protocol modifications.
	Samples contain analyte concentrations greater than highest standard point	Samples may require dilution and analysis.
High variation in samples and/or standards	Multichannel pipette errors	Confirm that pipette calibrations are accurate.
	Plate washing was not adequate or uniform	Ensure pipette tips are tightly secured. Ensure uniformity in all wash steps.
	Non-homogenous samples	Thoroughly mix samples before assaying.
	Samples may have high particulate matter	Remove particulate matter by centrifugation.
	Cross-well contamination	Do not reuse plate sealers. Always change tips for reagent additions. Ensure that pipette tips do not touch the reagents on the plate.

ELISA Plate Template

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

Legend Max™ Kits are manufactured by BioLegend Inc.
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