

Human Leptin

ELISA MAX™ Deluxe Set

Cat. No. 444304



BioLegend's ELISA MAX™ Deluxe Set contains the components necessary for the accurate quantification of natural and recombinant human Leptin protein. The set is designed for cost-effective and accurate quantification of human Leptin protein in serum, plasma, and cell culture supernatant. BioLegend's ELISA MAX™ Deluxe Sets are sensitive, accurate, and robust.

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

Materials Provided

- Human Leptin Capture Antibody (200X)
- Human Leptin Detection Antibody (200X)
- Human Leptin Standard
- Avidin-HRP (1000X)
- Substrate Solution F
- Coating Buffer B (5X)
- Assay Diluent B (5X)
- Blocking Buffer A (2X)

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.

Principle of the Test

BioLegend ELISA MAX™ Deluxe Set contains pre-optimized essential reagents and additional buffers and solutions for Sandwich ELISA assay.

A human Leptin specific capture antibody is first coated on a 96-well plate. Standards and samples are added to the wells, and human Leptin binds to the immobilized capture antibody. Next, a biotinylated anti-human Leptin detection antibody is added, producing an antibody-antigen-antibody "sandwich". The Avidin-HRP reagent is subsequently added, followed by TMB Substrate, producing a blue color in proportion to the concentration of human Leptin present in wells. Then Stop Solution should be added to wells to terminate the reaction. This step changes the reaction color from blue to yellow. The absorbance in wells should be read at 450nm and 570nm using a microplate reader.

For research purposes only. Not for use in diagnostic or therapeutic procedures.

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Materials to be Provided by the End-User

- Microwell plates: BioLegend Cat. No. 423501 is recommended
- Wash Buffer: BioLegend Cat. No. 421601 is recommended, or PBS + 0.05% Tween-20
- Stop Solution: BioLegend Cat. No. 423001 is recommended, or acid solution, e.g. 2N H₂SO₄
- Plate Sealers: BioLegend Cat. No. 423601 is recommended
- PBS (Phosphate-Buffered Saline): 8.0 g NaCl, 1.16 g Na₂HPO₄, 0.2 g KH₂PO₄, 0.2 g KCl, add deionized water to 1 L; pH to 7.4, 0.2 μm filtered
- Deionized (DI) water
- A microplate reader capable of measuring absorbance at 450/570 nm
- Adjustable pipettes to measure volumes ranging from 2 μL to 1 mL
- Wash bottle or automated microplate washer
- Log-Log graph paper or software for data analysis
- Tubes to prepare standard dilutions
- Timer

Storage Information

- Store kit components between 2°C and 8°C.
- After reconstitution of the lyophilized standard with 1X Assay Diluent B, aliquot into polypropylene vials and store at -70°C for up to one month. Avoid repeated freeze/thaw cycles.
- Prior to use, bring all components to room temperature (18°C-25°C). Upon assay completion return all components to appropriate storage conditions.

Health Hazard Warnings

- Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin. Refer to the MSDS online for details (<http://www.biolegend.com/msds>).
- Substrate Solution F is harmful if ingested. Additionally, avoid skin, eye or clothing contact.
- To reduce the likelihood of blood-borne transmission of infectious agents, handle all serum and/or plasma in accordance with NCCLS regulations.

Specimen Collection and Handling

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 sample in a citrate, heparin or EDTA containing tube. 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. Plasma specimens should be clear and non-hemolyzed.

Cell Culture Supernatant: If necessary, centrifuge to remove debris prior to analysis. Samples can be stored at < -70°C. Avoid repeated freeze/thaw cycles.

Reagent and Sample Preparation

Do not mix reagents from different sets or lots. Reagents and/or antibodies from different manufacturers should not be used with this set. All reagents should be diluted immediately prior to use.

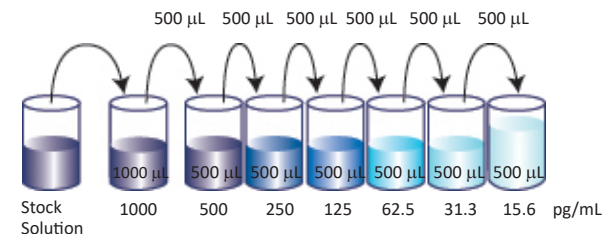
NOTE: Precipitation of 5X Assay Diluent B may be observed when stored long term between 2°C and 8°C. The precipitation does not alter the performance of the Buffer. If heavy precipitation is observed after the dilution to 1X Assay Diluent B, it can be filtered to clarify the solution.

Preparation of 1X Reagent for 1 Plate

Material	Dilute with
2.4 mL of Coating Buffer B (5X)	9.6 mL of Deionized Water
60 μL of Capture Antibody (200X)	12 mL of 1X Coating Buffer B
12 mL of Assay Diluent B (5X)	48 mL of PBS
60 μL of Detection Antibody (200X)	12 mL of 1X Assay Diluent B
12 μL of Avidin-HRP (1000X)	12 mL of 1X Assay Diluent B
12 mL Blocking Buffer A (2X)	12 mL of Deionized Water

Lyophilized vials are under vacuum pressure. Reconstitute the Human Leptin Standard by adding the volume of 1X Assay Diluent B to make the 50 ng/mL standard stock solution (Refer to ELISA MAX Kit Lot-Specific Certificate of Analysis/ELISA MAX Kit Protocol). Allow the reconstituted standard to sit for 15 minutes at room temperature, then mix gently prior to making dilutions.

Prior to use, prepare 1,000 μL of the top standard at a concentration of 1000 pg/mL from the stock solution in 1X Assay Diluent B. Perform six two-fold serial dilutions of the 1000 pg/mL top standard with 1X Assay Diluent B in separate tubes. After diluting, the Human Leptin standard concentrations are 1000 pg/mL, 500 pg/mL, 250 pg/mL, 125 pg/mL, 62.5 pg/mL, 31.3 pg/mL, and 15.6 pg/mL, respectively. 1X Assay Diluent B serves as the zero standard (0 pg/mL).



Samples: In general, serum and plasma samples require a 50-fold dilution. Cell culture supernatant samples require no dilution. If further dilution is required, samples should be diluted in 1X Assay Diluent B before adding to the wells.

Assay Procedure

Do not use sodium azide in any solutions as it inhibits the activity of the horseradish-peroxidase enzyme.

- One day prior to running the ELISA, dilute Capture Antibody in 1X Coating Buffer B as described in Reagent Preparation. Add 100 μL of this Capture Antibody solution to all wells of a 96-well plate. Seal plate and incubate overnight (16-18 hrs) between 2°C and 8°C.
- Bring all reagents to room temperature (RT) 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
- Wash plate 4 times with at least 300 μL Wash Buffer per well and blot residual buffer by firmly tapping plate upside down on absorbent paper. **All subsequent washes should be performed similarly.**
- To block non-specific binding and reduce background, add 200 μL 1X Blocking Buffer A per well.
- Seal plate and incubate at RT for 1 hour with shaking on a plate shaker (e.g. 500 rpm with a 0.3 cm circular orbit). All subsequent incubation with shaking should be performed similarly.

- While plate is being blocked, prepare standards and appropriate sample dilutions if necessary.
- Wash plate 4 times with Wash Buffer.
- Add 100 μ L of standards and samples to the appropriate wells. (If further dilution is required, samples should be diluted in 1X Assay Diluent B before adding to the wells.)
- Seal plate and incubate at RT for 2 hours with shaking.
- Wash plate 4 times with Wash Buffer.
- Add 100 μ L of diluted Detection Antibody solution to each well, seal plate and incubate at RT for 1 hour with shaking.
- Wash plate 4 times with Wash Buffer.
- Add 100 μ L of diluted Avidin-HRP solution to each well.
- Seal plate and incubate at RT for 30 minutes with shaking.
- Wash plate 5 times with Wash Buffer. For this final wash, soak wells in Wash Buffer for 30 seconds to 1 minute for each wash. This will help minimize background.
- Add 100 μ L of TMB Substrate Solution and incubate **in the dark** for 10 minutes*. Positive wells should turn blue in color. It is not necessary to seal the plate during this step.
- Stop reaction by adding 100 μ L of Stop Solution to each well. Positive wells should turn from blue to yellow.
- Read absorbance at 450 nm and 570 nm within 15 minutes, the absorbance at 570 nm can be subtracted from the absorbance at 450 nm.

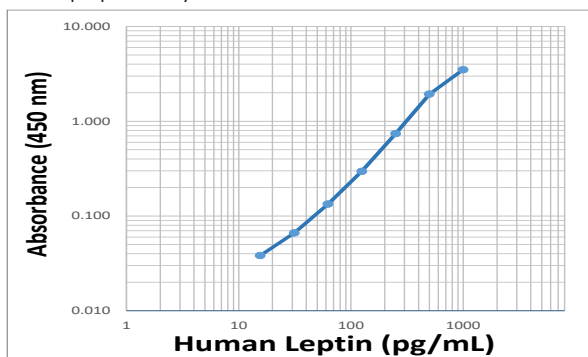
*Optimal substrate incubation time depends on laboratory conditions and the optical linear ranges of ELISA plate readers

Calculation of Results

Plot the standard curve on log-log axis 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 in the samples, find the absorbance value of the unknown 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 corresponding analyte concentration. If the samples were diluted, multiply by the appropriate dilution factor. The data is best calculated with computer-based curve-fitting software using a 5- or 4-parameter logistics curve-fitting algorithm. If a test sample's absorbance value falls outside the standard curve ranges, that test sample needs to be reanalyzed at a higher or lower dilution as appropriate.

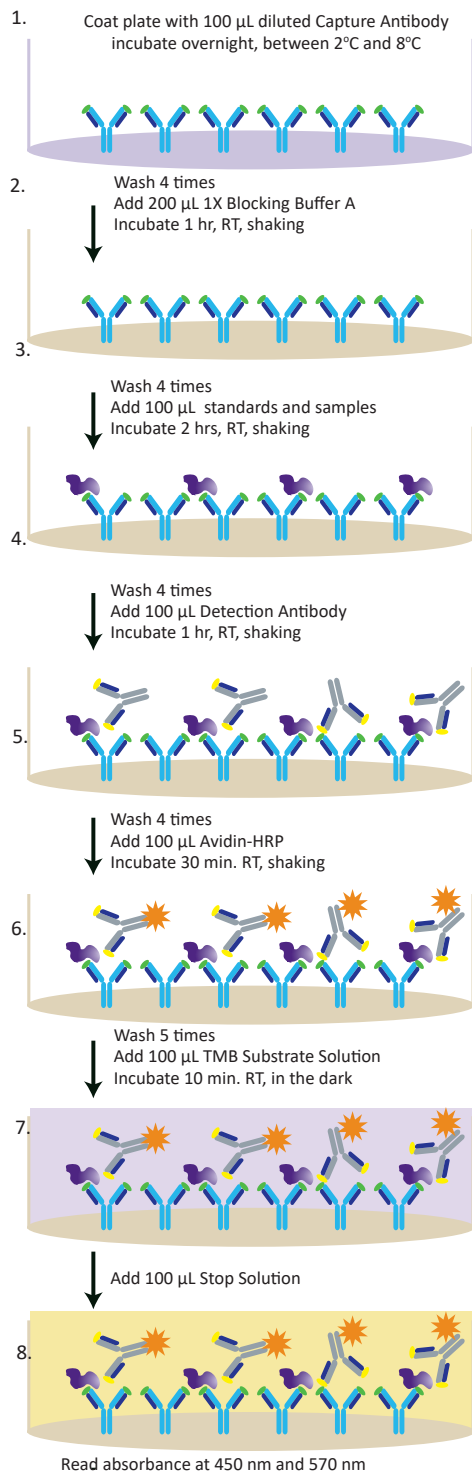
Typical Data

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



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Assay Procedure Summary



Performance Characteristics

Sensitivity: The expected minimum detectable concentration of human Leptin for this set is 4.7-16.3 pg/mL.

Specificity: No cross reactivity was observed when this kit was used to analyze multiple close-family human recombinant proteins, such as IL-6, IL-11, IL-12, and Oncostatin M.

Troubleshooting

High Background:

- Background wells were contaminated.
- Matrix used had endogenous analyte.
- Plate was insufficiently washed.
- TMB Substrate Solution was contaminated.

No signal:

- Incorrect or no antibodies were added.
- Avidin-HRP was not added.
- Substrate solution was not added.
- Wash buffer contains sodium azide.

Low or poor signal for the standard curve:

- Standard was incompletely reconstituted or was stored improperly.
- Reagents were added to wells with incorrect concentrations.
- Plate was incubated with improper temperature, timing or agitation.

Signal too high, standard curves saturated:

- Standard was reconstituted with less volume than required.
- One or more reagent incubation steps were too long.
- Plate was incubated with inappropriate temperature, timing, or agitation.

Sample readings out of range:

- Samples contain no or below detectable levels of analyte.
- Samples contain analyte concentrations greater than highest standard point.

High variations in samples and/or standards:

- Pipetting errors may have occurred.
- Plate washing was inadequate or nonuniform.
- Samples were not homogenous.
- Samples or standard wells were contaminated.

BioLegend, Inc.

BioLegend is ISO 13485 certified.

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For other technical resources, please visit:

<http://www.biolegend.com/en-us/support> or
email: techserv@biolegend.com