HUMAN LEPTIN ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF HUMAN LEPTIN CONCENTRATIONS IN CELL CULTURE SUPERNATES, SERUM, AND PLASMA

ALWAYS REFER TO LOT SPECIFIC PROTCOL PROVIDED WITH EACH KIT FOR INSTRUCTIONS. PROTOCOL MUST BE READ BEFORE USING THIS PRODUCT.

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

PRODUCT INFORMATION:

ELISA NAME	HUMAN LEPTIN ELISA
Catalog No.	SK00050-02
Lot No.	
Formulation	96 T
Standard range	31.2 - 2000 pg/mL
Sensitivity	15 pg/mL
Sample Volume	100 µl
Dilution factor	10 **(Optimal dilutions should be determined by each laboratory for each application)**
Sample Type	Serum, EDTA Plasma, Cell Culture Supernates
Specificity	Human Leptin only
Calibration	Human Leptin recombinant
Intra-assay Precision	4-8%
Inter-assay Precision	8-12%
Storage	2-8°C
This kit contains sufficient materials to run 35 samples duplicated provided that assay is run according to protocol.	

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DESCRIPTION

This Human Leptin ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and/or natural human Leptin from cell culture supernates, serum and plasma in a sandwich ELISA format.

This immunoassay contains recombinant human Leptin and antibodies raised against this protein. Results from this immunoassay have shown to accurately quantify recombinant and natural Leptin samples.

ASSAY OVERVIEW

This assay employs the quantitative sandwich ELISA format. The plate is pre-coated with an antibody specific for human Leptin. The capture antibody can bind to the human Leptin in the standard and samples. After washing the plate of any unbound substances, a biotinylated antibody against human Leptin is added to the wells. After another washing of the plate, Streptavidin-HRP Conjugate is added. After the last wash to remove any unbound enzyme, a substrate solution is added to the wells and color develops in direct proportion to the amount of human Leptin bound in the standard solutions or samples. A standard curve can be established and sample values can be read off the standard curve.

PROCEDURAL LIMITATIONS

_FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
_This ELISA kit should not be used beyond the expiration date on the kit label.
_Do not mix reagents with those from other lots or sources.
_It is important that the Dilution Buffer selected for the standard curve be consistent with the samples being assayed.
_Each laboratory must determine the optimal dilution factors for the samples being assayed with a pretest. If samples generate values that are not within the dynamic range of the standard curve, further concentrate or dilute the samples as required with Dilution Buffer and repeat the assay.
_Any modifications in buffers, pipetting technique, washing technique, incubation time or temperature, as well as kit age can cause a change in signal.
_Not all interfering factors have been tested in the immunoassay, therefore the possibility of interference cannot be excluded.

COMPONENTS PROVIDED

DESCRIPTION	CODE	QUANTITY
Leptin Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against Leptin.	050-02-01	1 plate
Leptin Standard – 2000 pg/vial of recombinant human Leptin in a buffered protein base with preservatives; lyophilized.	050-02-02	1 vial
Detection Antibody Concentrate – 1.05mL/vial, 10-fold concentrated of biotinylated monoclonal antibody against Leptin with preservatives; lyophilized.	050-02-03	1 vial
Positive Control – one vial of recombinant human Leptin; lyophilized.	050-02-04	1 vial
Streptavidin-HRP Conjugate - 60 μL/vial, 200-fold concentrated solution of Streptavidin conjugate to HRP with preservative.	SAHRP	1 vial
Dilution Buffer - 60mL of buffered protein based solution with preservative.	DB01	1 bottle
HRP Diluent Solution – 12mL of buffered protein based solution with preservative.	DB08	1 bottle
Wash Buffer - 50 mL of 10-fold concentrated buffered surfactant, with preservative.	WB01	1 bottle
TMB Substrate Solution - 11 mL of TMB substrate solution	TMB01	1 bottle
Stop Solution – 11 mL of 0.5M HCl	S-STOP	1 bottle
Plate Sealer	EAPS	1
Plastic Pouch	P01	1

STORAGE

Unopened Kit: Store at 2 - 8° C for up to 8 months. For longer storage, unopened Standard, Positive Control and Detection Antibody Concentrate should be stored at -20° C or -70° C. Do not use kit past expiration date.

Opened / Reconstituted Reagents: Reconstituted Standard (stock) and Detection Antibody concentrated solution SHOULD BE STORED at -20° C

or -70° C for up to one month. Streptavidin-HRP Conjugate 200-fold concentrated solution (protect from light) and other components may be stored at 2 - 8° C for up to 8 months.

Microplate Wells: Return unused wells to the plastic pouch with the desiccant pack. Microplate may be stored for up to 6 months at 2 - 8° C after opening.

ADDITIONAL MATERIALS REQUIRED

- Microplate reader capable of absorbance measurement at 450 nm.
- Microplate shaker (250 – 300 rpm).
- Microplate washer or manifold dispenser.
- 100 mL and 500 mL graduated cylinders.
- Multi-channel Pipette, Pipettes and pipette tips.
- Deionized or distilled water.

SAMPLE COLLECTION AND STORAGE

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.

Optional: Use Aprotinin (enzyme inhibitor) for ALL sample collection to prevent sample degradation. 0.5 TIU per ml of sample solution.

SAMPLE PREPARATION

Serum and plasma samples may need a 10-fold dilution. A suggested 10-fold dilution is 30 µL sample + 270 µL Dilution Buffer. **Optimal dilutions should be determined by each laboratory for each application**.
**Use polypropylene test tubes.**

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved.

Dilute 50 mL of Wash Buffer Concentrate into deionized or distilled water (450 mL) to prepare 500 mL of 1x Wash Buffer.

Leptin Standard - Reconstitute the Leptin standard with 1.0 mL of Dilution Buffer. This reconstitution produces a stock solution of 2000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 250 µL of Dilution Buffer into tubes #1 to #6. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The **2000 pg/mL** standard serves as the high standard. The Dilution Buffer serves as the zero standard (0 pg/mL).

Tube	Standard	Dilution Buffer	Concentration
stock	Powder	1000 µl	2000 pg/ml
# 1	250 µl of stock	250 µl	1000 pg/ml
# 2	250 µl of 1	250 µl	500 pg/ml
# 3	250 µl of 2	250 µl	250 pg/ml
# 4	250 µl of 3	250 µl	125 pg/ml
# 5	250 µl of 4	250 µl	62.5 pg/ml
# 6	250 µl of 5	250 µl	31.25 pg/ml

| Standard | Stock | 1 | 2 | 3 | 4 | 5 | 6 |
| Concentration | 2000 | 1000 | 500 | 250 | 125 | 62.5 | 31.2 | pg/ml |
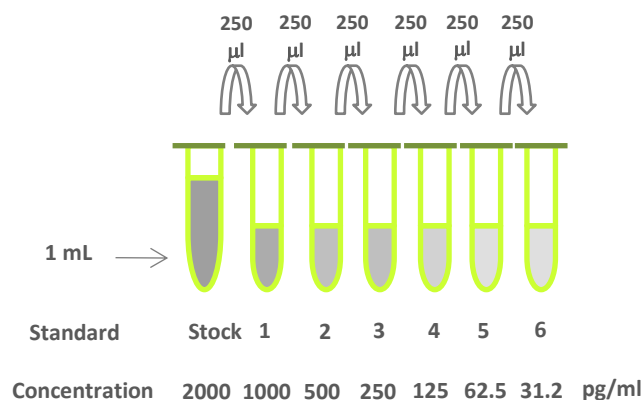
Positive Control - Reconstitute the positive control with 1.0 mL of Dilution Buffer to make positive control working solution. **Note:** Positive control working solution could be used within a few days (store at -20° C or -70° C).

Detection Antibody Concentrate - Reconstitute the Detection Antibody Concentrate with 1.05 mL of Dilution Buffer to produce a 10-fold concentrated stock solution. Pipette 9.45 mL of Dilution Buffer

into a 15 mL centrifuge tube and transfer 1.05 mL of 10-fold concentrated stock solution to prepare working solution.

Streptavidin-HRP Conjugate - Pipette 11.94 mL of **HRP Diluent Solution (DB08)** into a 15 mL centrifuge tube and transfer 60 μL of 200-fold concentrated stock solution to prepare working solution. **Note:** 1x working solution of Streptavidin-HRP conjugate should be used within a few days (protect from light).

ELISA PROTOCOL
Bring all reagents and samples to room temperature before the start of the assay. Blank, standard dilutions, positive control and samples should be assayed in duplicate. ELISA Protocol may need further optimization.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the plastic pouch with the desiccant pack.
3. Add 100 μL of Dilution Buffer to Blank wells.
4. Add 100 μL of Standard dilutions, sample, or positive control per well. Cover with plate sealer. Incubate for 2 hours on microplate shaker at room temperature.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with 1x Wash Buffer (300 μL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 100 μL of Detection Antibody working solution to each well. Cover with plate sealer. Incubate for 2 hours on microplate shaker at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 μL of Streptavidin-HRP Conjugate working solution to each well. Incubate for 60 minutes on microplate shaker at room temperature. **Protect from light**.
9. Repeat the aspiration/wash as in step 5.
10. Add 100 μL of Substrate Solution to each well. Incubate for 1-10 minutes on microplate shaker at room temperature. **Protect from light.**

11. Add 100 μL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green, or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
12. Determine the optical density of each well within 15 minutes, using a microplate reader set to 450 nm.

CALCULATION OF RESULTS
Average the duplicate readings for each standard, positive control and sample, and subtract the average zero standard optical density. Create a standard curve by reducing the data using computer software capable of generating a log-log curve fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the Leptin concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL DATA
This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

LEPTIN (PG/ML)	CORRECTED (450NM)
Blank	0 (0.053)
31.25	0.014
62.5	0.027
125	0.064
250	0.189
500	0.510
1000	1.274
2000	2.774

- **Lot No.:**
- **Positive Control :**

SPECIFICITY

PROTEIN	CROSSREACTIVITY (%)
Human Leptin	100
Mouse Leptin	0
Rat Leptin	0
Human Soluble l Leptin Receptor	0
Human Adiponectin	0
Human Resistin	0
Human SPARC	0

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
⬇
Add 100 µl of standard dilutions, samples, or positive control to each well. Incubate 2 hours on the plate shaker at RT.
⬇
Aspirate and wash 4 times.
⬇
Add 100 µl Detection Antibody working solution to each well. Incubate 2 hours on the plate shaker at RT.
⬇
Aspirate and wash 4 times.
⬇
Add 100 µl Streptavidin-HRP conjugate working solution to each well. Incubate 60 min on the plate shaker at RT. **Protect from light.**
⬇
Aspirate and wash 4 times.
⬇
Add 100 µl Substrate Solution to each well. Incubate 1-10 min on the plate shaker. **Protect from light.**
⬇
Add 100 µl Stop Solution to each well. Read 450nm within 15 min.