MOUSE/RAT LEPTIN ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF MOUSE/RAT LEPTIN CONCENTRATIONS IN CELL CULTURE SUPERNATES, SERUM, AND PLASMA

PRODUCT INFORMATION:

ELISA NAME	MOUSE/RAT LEPTIN ELISA
Catalog No.	SK00050-08
Lot No.	
Formulation	96 T
Standard range	125 - 8000 pg/mL
Sensitivity	30 pg/mL
Sample Volume	100 µL
Dilution factor	**Optimal dilutions should be determined by each laboratory for each application**
Sample Type	Serum, Plasma, Cell Culture Supernates
Specificity	Mouse/Rat Leptin
Calibration	Mouse 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.	

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.

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DESCRIPTION

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

This immunoassay contains recombinant mouse 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 mouse Leptin. The capture antibody can bind to the mouse Leptin in the standard and samples. After washing the plate of any unbound substances, a biotinylated antibody against mouse 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 (TMB) is added to the wells and color develops in direct proportion to the amount of 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.	**050-08-01**	**1 plate**
Leptin Standard – 8000 pg/vial of recombinant mouse Leptin in a buffered protein base with preservative; lyophilized.	**050-08-02**	**1 vial**
Detection Antibody Concentrate – 1.05 mL/vial, 10-fold concentrate of biotinylated antibody against Leptin with preservative; lyophilized.	**050-08-03**	**1 vial**
Positive Control – one vial of recombinant mouse Leptin; lyophilized.	**050-08-04**	**1 vial**
Streptavidin-HRP Conjugate - 120 µl/vial, 100-fold concentrated solution of Streptavidin conjugated to HRP with preservative.	**SAHRP**	**1 vial**
Dilution Buffer – 60 mL of buffered protein based solution with preservative.	**DB01**	**1 bottle**
HRP Diluent Solution – 12 mL 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 piece**
Plastic Pouch	**P01**	**1 piece**

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) solution and Detection Antibody

concentrated solution SHOULD BE STORED at -20° C or -70° C for up to one month. Streptavidin-HRP Conjugate 100-fold concentrated solution and TMB Substrate Solution can be stored at 2 – 8° C for up to 8 months (**DO NOT FREEZE** and **PROTECT FROM LIGHT**). All other components may be stored at 2 – 8° C for up to 8 months.
Microplate Wells: Return unused strips 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.

PRECAUTION

This kit should be handled by those persons who have been trained in and can follow the principles of good laboratory practice. Wear protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken while handling solutions in this kit to avoid contact with skin or eyes, especially with the stop solution because it contains diluted hydrochloric acid. Wash immediately with water in case of contact on skin or eyes.

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

Mouse or rat serum and plasma samples DO NOT need to be diluted, but if samples are over the highest standard, then a 2-fold or greater dilution is needed. A suggested 2-fold dilution is 125 µL sample + 125 µL Dilution Buffer. **Optimal dilutions should be determined by each laboratory for each application**.
Use polypropylene test tubes.

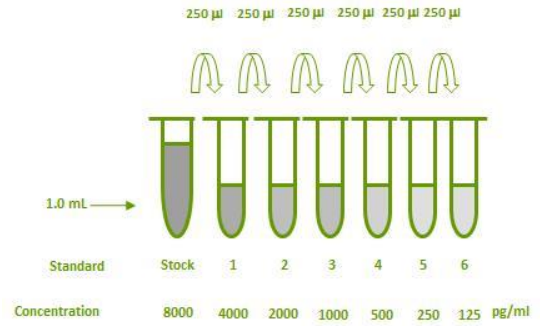
REAGENT PREPARATION

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 mL of Dilution Buffer. This reconstitution produces a stock solution of 8000 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 **8000 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	1.0mL	8000 pg/ml
# 1	250 µl of stock	250 µl	4000 pg/ml
# 2	250 µl of 1	250 µl	2000 pg/ml
# 3	250 µl of 2	250 µl	1000 pg/ml
# 4	250 µl of 3	250 µl	500 pg/ml
# 5	250 µl of 4	250 µl	250 pg/ml
# 6	250 µl of 5	250 µl	125 pg/ml



Positive Control - Reconstitute the positive control with 1 mL of Dilution Buffer to make positive control working solution. **Note:** Positive control working solution could be reused within a few days if stored at -20° C ~ -70° C.

Detection Antibody - 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 11.88 mL of **HRP Diluent Solution (DB08)** into a 15 mL centrifuge tube and transfer 120 µL of 100-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 per well 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 45 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 2-10 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 STANDARD CURVE

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

LEPTIN (PG/ML)	CORRECTED (450NM)
Blank	0 (0.067)
62.5 (optional)	0.072
125	0.120
250	0.218
500	0.390
1000	0.631
2000	0.870
4000	1.154
8000	1.429

- Lot No.:
- Positive Control :

SPECIFICITY

This assay recognizes both natural and recombinant mouse Leptin. Data also indicates that rat serum and plasma samples were bound to the antibody that was used in this kit formulation condition. Its linear dilution curves were parallel to the standard curves obtained using the ELISA standard. This means rat serum and plasma samples cross-react with mouse Leptin ELISA kit. There is no cross-reaction with human Leptin.

LINEARITY

To assess the linearity of the assay pooled research mouse serum samples were diluted with Dilution Buffer (DB01) and assayed.

DILUTION FACTOR	ASSAYED (PG/ML)	FINAL (PG/ML)	RECOVERY (%)
5 X	1065.586	5327.93	100
10 X	549.595	5495.95	103

To assess the linearity of the assay pooled research mouse EDTA plasma samples were diluted with Dilution Buffer (DB01) and assayed.

DILUTION FACTOR	ASSAYED (PG/ML)	FINAL (PG/ML)	RECOVERY (%)
5 X	1021.367	5106.835	100
10 X	629.790	6297.90	123

To assess the linearity of the assay pooled research rat serum samples were diluted with Dilution Buffer (DB01) and assayed.

DILUTION FACTOR	ASSAYED (PG/ML)	FINAL (PG/ML)	RECOVERY (%)
1 X	1048.566	1048.566	100
2 X	572.944	1145.888	110
4 X	327.825	1311.3	125

To assess the linearity of the assay pooled research rat EDTA plasma samples were diluted with Dilution Buffer (DB01) and assayed.

DILUTION FACTOR	ASSAYED (PG/ML)	FINAL (PG/ML)	RECOVERY (%)
1 X	818.092	818.092	100
2 X	373.860	753.72	92.1
4 X	242.901	971.604	119

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
⬇
Add 100 µl of standard dilutions, samples, or positive control to the 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 45 min on the plate shaker at RT. **Protect from light**.
⬇
Aspirate and wash 4 times.
⬇
Add 100 µl Substrate Solution to each well. Incubate 2-10 min on plate shaker at RT. **Protect from light**.
⬇
Add 100 µl Stop Solution to each well. Read 450nm within 15 min.