PURCHASE INFORMATION:

ELISA NAME	**MOUSE/RAT LEPTIN ELISA**
Catalog No.	
Lot No.	
Formulation	
Standard range	
Sensitivity	
Sample Volume	
Dilution factor	
Sample Type	
Specificity	
Intra-assay Precision	
Inter-assay Precision	
Storage	

Mouse/Rat LEPTIN immunoassay is a 3.5 - 4.5 hour solid phase ELISA designed to measure Mouse and Rat LEPTIN in cell culture supernates, serum, and plasma. It contains recombinant mouse LEPTIN and antibodies raised against this protein. It has been shown to accurately quantify recombinant mouse LEPTIN. Results obtained with naturally occurring LEPTIN samples showed linear curves that were parallel to the standard curves obtained using the kit standards. These results indicate that the immunoassay kit can be used to determine relative mass values for natural Mouse or Rat LEPTIN.

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for LEPTIN has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any LEPTIN present is bound by the immobilized antibody. After washing away any unbound substances, a biotinylated antibody specific for LEPTIN is added to the wells. Following a wash to remove any unbound antibody-biotin reagent, HRP link Streptavidin is added to the wells. After washing away any unbound enzyme, a substrate solution is added to the wells and color develops in proportion to the amount of LEPTIN bound in the initial step. The color development is stopped and the intensity of the color is measured.

_ FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
_ The kit should not be used beyond the expiration date on the kit label.
_ Do not mix or substitute 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.
_ If samples generate values higher than the highest standard, dilute the samples with Dilution Buffer and repeat the assay.
_ Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
_ This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the immunoassay, the possibility of interference cannot be excluded.

DESCRIPTION	CODE	QUANTITY
Leptin Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against mouse LEPTIN.	**050-08-01**	**1 plate**
LEPTIN Standard – 8000 pg/vial of recombinant mouse LEPTIN in a buffered protein base with preservatives; lyophilized.	**050-08-02**	**1 vial**
Detection Antibody Concentrate – 105 µL/vial, 100-fold concentrated of biotinylated antibody against LEPTIN with preservatives; lyophilized.	**050-08-03**	**1 vial**
Positive Control – one vial of recombinant mouse LEPTIN, lyophilized	**050-08-04**	**1 vial**
Streptavidin-HRP Conjugate - 60 ul/vial, 200-fold concentrated solution of Streptavidin conjugate to HRP with preservatives	**SAHRP**	**1 vial**
Dilution Buffer - 60mL of buffered protein based solution with preservatives	**DB01**	**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**

Unopened Kit: Store at 2 - 8°C for up to 6 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, Detection Antibody Solution SHOULD BE STORED at -20°C or – 70°C for up to one month. Streptavidin-HRP Conjugate 200-fold Concentrate

and other components may be stored at 2 - 8°C for up to 6 months.
Microplate Wells: Return unused wells to the plastic bag containing the desiccant pack, reseal along entire edge of zip-seal. Microplate may be stored for up to 6 months at 2 - 8°C.

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Microplate shaker (250-300rpm).
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 100 mL and 500 mL graduated cylinders.

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.
Note: Use Aprotinin (enzyme inhibitor) (Code No.: 00700-01-25) for ALL sample collection to prevent sample degradation. 0.5 TIU per ml of sample solution.

Mouse serum and plasma samples may need a 5-10-fold dilution. A suggested 5-fold dilution is 50 µL sample + 200 µL Dilution Buffer. A suggested 10-fold dilution is 25 µL sample + 225 µL Dilution Buffer. Rat serum and plasma samples may not need to be diluted. **Optimal dilutions should be determined by each laboratory for each application**.
Use polypropylene test tubes.

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 Wash Buffer.

LEPTIN Standard - Refer to vial label for reconstitution volume. Reconstitute the **LEPTIN** Standard with 1.0 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).

250 µl	2 µl	250 µl	2 µl	2 µl	250 µl	

1 mL							
Standard	ock	1	2	3	4	5	6
Concent	80	40	200	10	50	250	125

Detection Antibody - Reconstitute the **Detection Antibody Concentrated** with 105 µl of Dilution Buffer to produce a 100-fold concentrated stock solution. Pipette 10.395 mL of Dilution Buffer into a 15 ml centrifuge tube and transfer 105 µl of 100-fold

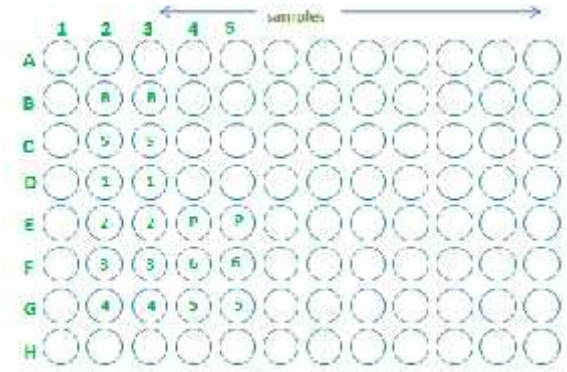
concentrated stock solution to prepare working solution.

Streptavidin-HRP Conjugate - Pipette 11.94 mL of Dilution Buffer 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.

Positive Control - Reconstitute the positive control with 1.0 mL of Dilution Buffer to make positive control working solution. **Note:** Positive control working solution should be used within a few days.

Bring all reagents and samples to room temperature before use. It is recommended that standards be assayed in duplicates.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess micro-plate strips from the plate frame, return them to the plastic bag containing the desiccant pack, reseal.
3. Add 100 µL of Dilution Buffer to Blank wells (B2, B3).
4. Add 100 µL of Standard (C2, C3 to G2, G3 and F4, F5 to G4, G5), sample, or positive control (E4, E5) per well. Cover with plate sealer. Incubate for 2 hours on micro-plate shaker at room temperature. A plate layout is provided to record standards and samples assayed.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with 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 sealer. Incubate for 2 hours on micro-plate 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 micro-plate 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 8-12 minutes 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 micro-plate reader set to 450 nm.



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.

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

This immunoassay is calibrated against a highly purified E. Coli-expressed recombinant mouse LEPTIN.

Twenty-five assays were evaluated and the minimum detectable dose (MDD) of LEPTIN was 62.5 pg/mL.

This assay recognizes both natural and recombinant mouse or rat LEPTIN. That elisa kit does not crossreact with soluble Leptin receptor.

Add 100μl of standard, samples, 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 8-12 min on plate shaker. **Protect from light.**

Add 100 μl Stop Solution to each well. Read 450nm within 15 min