Mouse Leptin ELISA Kit

1. Catalog No. K0331250

2. Quantity 96 tests

3. Storage 4°C

4. Description Mouse Leptin ELISA kit contains all the necessary reagents required for performing quantitative measurement of Mouse Leptin levels from samples including serum, plasma, culture medium or other biological fluids in a sandwich ELISA format.

5. Standard curve range 20-1250 pg/ml

6. Kit Contents

Component	Description	Amount
Pre-Coated 96 well ELISA microplate	Antigen-affinity purified Rabbit anti-Mouse Leptin pre-coated 96well plate	1 Plate
Detection Antibody (Lyophilized)	Biotinylated antigen-affinity purified Rabbit anti-Mouse Leptin	1 EA
Standard Protein (Lyophilized)	Recombinant Mouse Leptin	1 EA
Color Development Enzyme (Lyophilized)	Avidin-HRP conjugate	1 EA
Assay Diluent	0.1% Casein in PBS (50 ml)	1 EA
Color development Reagent A	TMB solution (10 ml)	1 EA
Color development Reagent B	Substrate(H_2O_2) solution (20 ml)	1 EA
Stop Solution	2M H_2SO_4 (10 ml)	1 EA
PBS powder	Pouch for 1 L	1 EA
Tween-20 (50%)	1 ml	1 EA
Plate Sealer		3 EA

7. Reconstitution & Storage

1. Mouse Leptin Standard: 100 ng (1 vial) of recombinant Mouse Leptin should be reconstituted in 0.1 ml sterile water for a concentration of 1.0 ug/ml.
2. Detection Antibody: 10 ug (1 vial) of biotinylated antigen-affinity purified anti-Mouse Leptin should be reconstituted in 0.25 ml sterile water for a concentration of 40 ug/ml.
3. Color Development Enzyme: Avidin-HRP should be reconstituted in 60 ul sterile water.

Note: Reconstituted solutions are stable at -20°C for up to 2 months. Do not repeat frozen and thawing.

8. Reagent Preparations

*** All preparations should be mixed thoroughly and warmed up at room temperature prior to use.**

1. Washing Solution (PBST): Resolve the PBS powder (1 pouch) to sterile water and make 1 Liter, then add 1 ml Tween-20 (50%) to this solution and mix well.
2. Pre-coated ELISA 96 well plate: Select the number of coated wells required for the assay. The remaining wells should be placed in the resealable pouch with a desiccant. The pouch must be resealed to protect from moisture.

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3. Sample dilution: Dilute the samples to a proper concentration in Assay Diluent.
4. Standards
   Dilute the standards and samples in Assay Diluent at 1:2 serial dilutions as follows:

Step	Dilution Method	Standard conc.
Step A	2.5 ul of Standard + 2 ml of Assay Diluent	1250 pg/ml
Step B	1 ml of Step A + 1 ml of Assay Diluent	625 pg/ml
Step C	1 ml of Step B + 1 ml of Assay Diluent	312.5 pg/ml
Step D	1 ml of Step C + 1 ml of Assay Diluent	156.25 pg/ml
Step E	1 ml of Step D + 1 ml of Assay Diluent	78.125 pg/ml
Step F	1 ml of Step E + 1 ml of Assay Diluent	39.0625 pg/ml
Step G	1 ml of Step F + 1 ml of Assay Diluent	19.53 pg/ml

Note: Dilute the samples, based on the expected concentration of the analyte, to fall within the concentration range of the standards.

5. Detection Antibody: Dilute the reconstituted detection antibody in Assay Diluent to a concentration of 1 ug/ml (1/40 dilution).
6. Color Development Enzyme: Dilute the Avidin-HRP conjugate 1:300 in Assay Diluent.
7. Color development solution: Mix 1 volume of color development reagent A and 2 volume of reagent B (1:2) prior to use.

9. ELISA Protocol

1. Add 200 ul of Washing Solution to each well. Aspirate the wells to remove liquid and wash the plate 3 times using 300 ul of Washing Solution per well. After the last wash, invert plate to remove residual solution and blot on paper towel.
   Note: Do not dry the well completely and so immediately go on next step.
2. Add 100 ul of standard or sample to each well in duplicate. Cover with the Plate Sealer provided. Incubate at room temperature for at least 2 hours.
3. Aspirate the wells to remove liquid and wash the plate 4 times like as step 1.
4. Add 100 ul of the diluted detection antibody (1 ug/ml) per well. Cover with the Plate Sealer provided. Incubate at room temperature for 2 hours.
5. Aspirate and wash plate 4 times like as step 1.
6. Add 100 ul of the diluted Color Development Enzyme (1/300 dilute) per well. Cover with the Plate Sealer provided. Incubate 30 minutes at room temperature (or 37°C for 30 minutes).
7. Aspirate and wash plate 4 times like as step 1.
8. Add 100 ul of color development solution to each well. Incubate at room temperature for a proper color development. (3-30 minutes) To stop the color reaction, add 100 ul of the stop solution to each well.
9. Using a microtiter plate reader, read the plate at 450 nm wavelength.

10. Calculation of Results

1. Average the duplicate readings from each standard, control, and samples.
2. Subtract the zero reading from each averaged value above.
3. Create a standard curve by reducing the data using ELISA reader's computer software capable of generating Standard curve-fit.

* A standard curve should be generated for each set of samples (See example).

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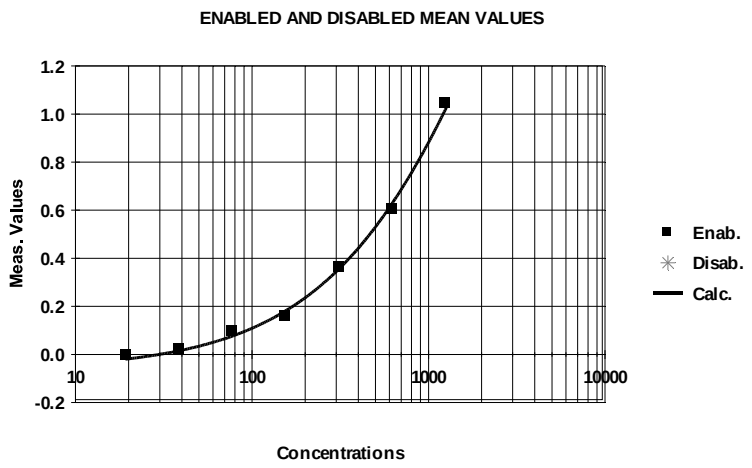

Mouse Leptin (pg/ml)

11. Troubleshooting

Problem	Solution
Low O.D.	1. Incorrect dilutions or pipetting errors 2. Improper incubation times 3. Improper mixing of the TMB substrate. Each component is mixed in equal parts. 4. Wrong filter on microtiter reader. Wavelength should be 450 nm for TMB, 490 nm for OPD, or 405 nm for ABTS. 5. Kit materials or reagents are contaminated or expired. 6. Incorrect reagents used.
High O.D.	1. Cross contamination from other samples or control 2. Incorrect dilutions or pipetting errors 3. Improper washing 4. Wrong filter on microtiter reader. Wavelength should be 450 nm for TMB, 490 nm for OPD, or 405 nm for ABTS. 5. Contaminated buffers or enzyme substrate 6. Improper incubation times 7. Kit materials or reagents are contaminated or expired.
Poor Duplicates	1. Poor mixing of specimens 2. Incorrect dilutions or pipetting errors 3. Inconsistency in following ELISA protocol 4. Inefficient washing
High Background	1. Contaminated buffers or enzyme substrate 2. Incorrect dilutions or pipetting errors 3. Kit materials or reagents are contaminated or expired. 4. Inefficient washing
None Development	1. Procedure not followed correctly 2. Contaminated buffers, enzyme substrate or conjugate 3. Kit materials or reagents are contaminated or expired.

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12. Cautions

1. Store all solutions at 4°C and keep them from contamination.
2. All samples and kit reagents should be at room temperature (20-25°C) prior to use.
3. Vigorous washing of the plate after incubation steps is essential to obtaining low background values.
4. Dissolve antigen, standard and antibody perfectly.
5. Use clean pipet tips for each transfer to avoid cross contamination.
6. Stop solution (H_2SO_4) is a caustic material. Eye, hand, face, and clothing protection should be worn when handling this material.
7. Individual components of this kit contain no preservatives.

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