KAMIYA BIOMEDICAL COMPANY

Mouse Leptin ELISA

For the quantitative determination of Leptin in mouse serum.

Cat. No. KT-380

For Research Use Only.

PRODUCT INFORMATION

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INTENDED USE
The Mouse Leptin ELISA is for the quantitative determination of Leptin in mouse serum. For research use only.

INTRODUCTION
Leptin, which is a product of the *ob* gene, is a protein consisting of 146 amino acids and is secreted from white adipose tissue. It is known that Leptin acts on the hypothalamus to decrease food intake and to reduce body weight, body fat, blood sugar and blood insulin in healthy and *ob/ob* mice. Further, gene expression of neuropeptide Y (NPY) is suppressed by Leptin. Recently, radioimmunoassays for Leptin determination in human plasma have become available and Leptin levels in a human patient group with obesity was found to increase in comparison with that of a normal group. The Leptin plasma levels correlated with body fat. These observations clearly show that Leptin concentration in human plasma reflects the weight of tissue fat. Therefore, the measurement of plasma or serum Leptin may be a good index of obesity. Although mouse Leptin shows a high homology (96%) with rat Leptin, it is observed that substitution of several amino acid residues occurs between human and mouse Leptin. These findings support the need of a highly sensitive immuno-assay system specific to mouse Leptin. Advantages of this assay include sensitive quantification, high specificity, no interference from other sample components, and no sample pretreatment. The Calibrator is a recombinant mouse Leptin.

PRINCIPLE
This kit is based on a sandwich enzyme immuno-assay. The 96-well plate is coated with highly purified anti-mouse Leptin antibody and Leptin Calibrator or sample is added for the first step of the immuno-reaction. After incubation and plate washing, biotinylated rabbit anti-mouse Leptin antibody is added to form Leptin antibody-antigen-biotinylated Leptin antibody complexes on the surface of the wells. After incubation and washing, HRP-Labeled streptoavidin (SA) is added to bind to the biotinylated Leptin antibody. Finally, HRP enzyme activity is determined by 3,3',5,5'-tetramethylbenzidine (TMB) and the concentration of mouse Leptin is calculated.

COMPONENTS

Component	Form	Quantity	Main Ingredient
1. Antibody-Coated Plate	Microtiter Plate	1 plate (96-well)	Rabbit anti-mouse Leptin antibody
2. Leptin Calibrator	Lyophilized	1 vial (20 ng)	Recombinant mouse Leptin
3. Labeled Antibody Solution	Liquid	1 bottle (12 mL)	Biotinylated rabbit anti-mouse Leptin pAb
4. SA-HRP Solution	Liquid	1 bottle (12 mL)	HRP-labeled SA
5. TMB Substrate Solution	Liquid	1 bottle (12 mL)	3,3',5,5'-tetramethylbenzidine (TMB)
6. Stop Solution	Liquid	1 bottle (12 mL)	1 M H_2SO_4
7. Buffer Solution	Liquid	1 bottle (15 mL)	Phosphate buffer
8. Wash Solution Concentrate	Liquid	1 bottle (50 mL)	Concentrated saline
9. Plate Seal		3 sheets	

MATERIALS REQUIRED BUT NOT PROVIDED
- Photometer for microtiter plate (plate reader), which can read absorbance up to 2.5 at 450 nm
- Rotator for microtiter plate
- Washing device for microtiter plate with aspiration system and dispenser
- Micropipettes, multi-channel pipettes for 8 wells or 12 wells and their tips
- Polypropylene or glass test tubes for preparation of Calibrator Solution
- Graduated cylinder (1,000 mL)
- Distilled water or de-ionized water

PRECAUTIONS

Protect reagents from strong light (e.g. direct sunlight) during storage and assay.

Satisfactory performance of the test is guaranteed only when reagents are used from a kit with identical lot number.

As pipetting operations may affect the precision of the assay, precisely pipette the prepared Calibrator Solutions or samples into corresponding wells. Use a new tip for each sample and calibrator to avoid cross-contamination. Use clean test tubes or vessels.

Always run a calibration curve when testing samples.

REAGENT PREPARATION

1. Preparation of Calibrator Solutions: Reconstitute the Leptin Calibrator (lyophilized Mouse Leptin, 20 ng/vial) with 1 mL of Buffer Solution, giving a 20 ng/mL Calibrator Solution after reconstitution. 0.2 mL of the reconstituted Calibrator Solution is diluted with 0.2 mL of Buffer Solution to yield a 10 ng/mL Calibrator Solution. Repeat the serial dilution to make Calibrator Solutions at 5, 2.5, 1.25, 0.625, and 0.313 ng/mL. Buffer Solution is used as the zero calibrator (0 ng/mL).

   Note: Calibrator Solution must be prepared immediately before assay. Use clean test tubes or vessels.
   In the case of multiple assays, the prepared Calibrator Solutions should be store at < -30°C.

   When a sample is predicted to be below 0.313 ng/mL, another calibrator may be prepared to decrease the lower limit of detection. Continue the serial dilution by diluting the 0.313 ng/mL Calibrator Solution to make a 0.156 ng/mL Calibrator Solution. However, the assay precision below 0.313 ng/mL may not be as good as the assay precision between 0.313 and 20 ng/mL.

2. Preparation of Wash Solution: Dilute 50 mL of Wash Solution Concentrate to 1,000 mL with distilled or de-ionized water. Diluted Wash Solution is stable for 6 months at 4°C.

   Note: During storage of the Wash Solution Concentrate at 4°C, precipitates may be observed, however, they will dissolve when diluted.

3. Other reagents are ready for use.

STORAGE

Store kit at 4°C.

SPECIMEN COLLECTION AND HANDLING

Serum samples must be used as soon as possible after collection. If the samples are to be tested at a later time, they should be divided into test tubes in small amounts and frozen below –30°C. Avoid repeated freeze/thaw cycles.

ASSAY PROTOCOL

1. Warm the reagents and samples to room temperature (20-30°C) at least 1 hour before beginning the test.
2. Add 0.35 mL of diluted Wash Solution into wells and aspirate the solution. Repeat this washing procedure twice for a total of 3 wash steps. Finally, invert the plate and tap onto an absorbent surface, such as paper toweling, to ensure removal of most of the residual Wash Solution.
3. Add 45 µL of Buffer Solution into each of the wells. Then add 25 µL of the prepared Calibrator Solutions (0, 0.313, 0.625, 1.25, 2.5, 5, 10, and 20 ng/mL) or samples into the wells.
4. Cover the plate with the Plate Seal and incubate at room temperature for 3 hours. During incubation, the plate should be rotated on a plate rotator.
5. Remove the Plate Seal and aspirate the solution in the wells. Wash the wells 4 times with approximately 0.35 mL/well of diluted Wash Solution. Finally, invert the plate and tap onto an absorbent surface, such as paper toweling, to ensure removal of most of the residual Wash Solution.
6. Pipette 100 µL of Labeled Antibody Solution into each of the wells.
7. Cover the plate with a Plate Seal and incubate at room temperature for 2 hours. During the incubation, the plate should be rotated on a plate rotator.

8. Remove the Plate Seal and aspirate the solution in the wells. Wash the wells 4 times with approximately 0.35 mL/well of diluted Wash Solution. Finally, invert the plate and tap onto an absorbent surface, such as paper toweling, to ensure removal of most of the residual Wash Solution.
9. Add 100 µL of SA-HRP solution into each of the wells.
10. Cover the plate with the Plate Seal and incubate at room temperature for 1 hour. During incubation, the plate should be rotated on a plate rotator.
11. Transfer the required volume of the TMB Substrate Solution into a vessel, and bring to room temperature protected from light for 1 hour before use. Store the remaining TMB Substrate Solution at 4°C.
12. Remove the Plate Seal and aspirate the solution in the wells. Wash the wells 4 times with approximately 0.35 mL/well of diluted Wash Solution. Finally, invert the plate and tap onto an absorbent surface, such as paper toweling, to ensure removal of most of the residual Wash Solution.
13. Add 100 µL of TMB Substrate Solution into each well, cover the plate with a Plate Seal and incubate for 30 minutes at room temperature, protected from light.
14. Add 100 µL of Stop Solution into the wells to stop the reaction.
15. Read the optical absorbance of the wells at 450 nm. The optical absorbance of reaction solution in wells should be read as soon as possible after stopping the color reaction.

Note: Perform all determinations in duplicate.

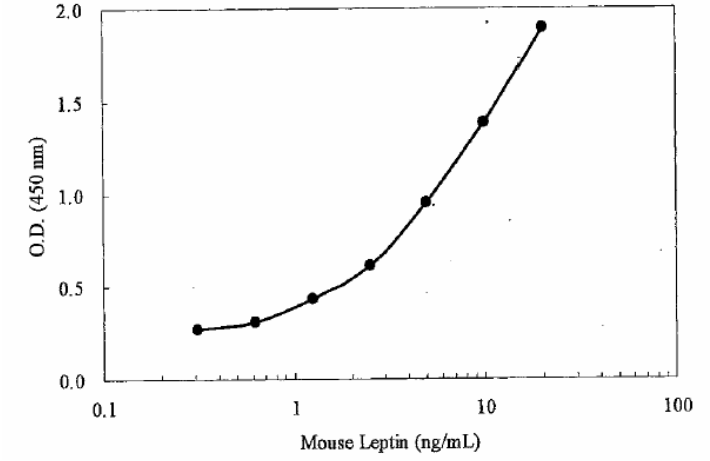
RESULTS

Calculate mean absorbance values of wells containing the Calibrators and plot a calibration curve on semilogarithmic graph paper (abscissa: concentration of Calibrators; ordinate: absorbance values of Calibrators). Use the calibration curve to read Leptin concentrations in samples from the corresponding absorbance values.

When the concentration of Leptin is expected to exceed 20 ng/mL, the sample must be diluted with Buffer Solution until the result is within the assay range.

PERFORMANCE

Typical Calibration Curve (example only, a new calibration curve for each run must be established by the end-user)



Analytical Recovery

Sample	Mouse Leptin Added (ng/mL)	Observed (ng/mL)	Expected (ng/mL)	Recovery (%)
Mouse Serum 1	0.0	1.69		
	0.3	2.17	1.99	109.05
	3.0	4.49	4.69	95.74
	7.0	8.48	8.69	97.58
Mouse Serum 2	0.0	2.11		
	0.3	2.52	2.41	104.56
	3.0	5.33	5.11	104.31
	7.0	8.41	9.11	92.32
Mouse Serum 3	0.0	1.20		
	0.3	1.58	1.50	105.33
	3.0	3.90	4.20	92.86
	7.0	8.91	8.20	108.66
Mouse Serum 4	0.0	0.86		
	0.3	1.02	1.16	87.93
	3.0	3.56	3.86	92.23
	7.0	6.84	7.86	87.02

Precision and reproducibility

- Intra-assay CV (%) Serum 5.01 – 9.84
- Inter-assay CV (%) Serum 4.37 – 7.71

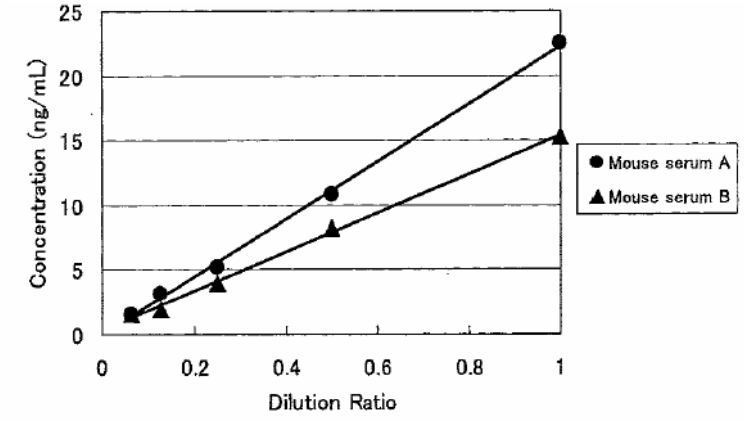
Assay Range

0.313 – 20 ng/mL

Cross-Reactivity

Mouse Leptin: 100%
Human Leptin: 0%
Rat Leptin: 33.8%

Dilution Test



FOR RESEARCH USE ONLY

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