KAMIYA BIOMEDICAL COMPANY

High Sensitive Rat Leptin ELISA

For the high sensitive quantitative determination of Leptin in rat serum or plasma.

Cat. No. KT-379

For Research Use Only.

PRODUCT INFORMATION

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INTENDED USE
The High Sensitive Rat Leptin ELISA is for the quantitative determination of Leptin in rat serum or plasma. For research use only.

INTRODUCTION
Leptin, which is a product of the *ob* gene, is a protein consisting of 167 amino acids and it is secreted from white adipose tissue. It is known that leptin acts on 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 levels correlate well with body fat. These observations clearly show that leptin concentration in human plasma reflects the weight of tissue fat. Therefore, the measurement of plasma leptin may be an excellent index of obesity. Although rat leptin shows a high homology (96%) with mouse leptin, it is observed that substitution of several amino acid residues occurs at both N- and C- terminal end regions between human and rat leptin. These findings support the need of a highly sensitive immuno-assay system specific to rat leptin. Advanages of this assay include sensitive quantification, high specificity, no cross-reactivity with other sample components, and no sample pretreatment. The Calibrator is a recombinant Rat Leptin.

PRINCIPLE
This kit is based on a two-step sandwich enzyme immuno-assay. The 96-well plate is coated with anti-rat leptin monoclonal antibody. Rat Leptin Calibrators or samples and Rabbit Polyclonal Antibody are added to the wells for the immuno-reaction. During this immunoreaction, monoclonal antibody-antigen-polyclonal antibody complex are formed on the surface of the wells. After incubation and plate washing, HRP-Labeled Antibody (goat anti-rabbit IgG-HRP conjugated) is added to bind to Rabbit Polyclonal Antibody. Finally, HRP enzyme activity is determined by o-Phenylenediamine dihydro-chloride (OPD) and the concentration of rat leptin is calculated.

COMPONENTS

Component	Form	Quantity	Main Ingredient
1. Antibody-Coated Plate	MTP[*1]	1 plate (96-well)	Anti-Rat Leptin mAb
2. Leptin Calibrator	Lyophilized	1 vial (20 ng)	Recombinant Rat Leptin
3. Rabbit Polyclonal Ab	Lyophilized	1 bottle	Rabbit anti-Rat Leptin pAb
4. HRP-Labeled Ab	Liquid	1 bottle (12 mL)	Goat anti-rabbit IgG-HRP conjugated
5. Substrate Buffer	Liquid	1 bottle (26 mL)	0.015% Hydrogen peroxide
6. OPD Tablet	Tablet	2 tablets	o-Phenylenediamine dihydrochloride
7. Stop Solution	Liquid	1 bottle (12 mL)	1 M H_2SO_4
8. Buffer Solution	Liquid	1 bottle (20 mL)	Tris-HCl buffer
9. Wash Solution Concentrate	Liquid	1 bottle (50 mL)	Concentrated saline
10. Plate Seal		4 sheets	

MTP[*1] ..... Microtiter plate

MATERIALS REQUIRED BUT NOT PROVIDED

- Photometer for microtiter plate (plate reader), which can read absorbance up to 2.5 at 492 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
- 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 Rat Leptin, 20 ng/vial) with 2 mL of Buffer Solution, giving a 10,000 pg/mL Calibrator Solution after reconstitution. 0.1 mL of the reconstituted Calibrator Solution is diluted with 0.3 mL of Buffer Solution to yield a 2,500 pg/mL Calibrator Solution. Then, 0.2 mL of the 2,500 pg/mL Calibrator Solution is diluted with 0.2 mL of Buffer Solution to yield a 1,250 pg/mL Calibrator Solution. Repeat the serial dilution to make Calibrator Solutions at 625, 312.5, 156.2, 78.1 pg/mL. Buffer Solution is used as the zero calibrator (0 pg/mL).

   Note: Calibrator Solution must be prepared immediately before assay. Use clean test tubes or vessels.
   In the case of multiple assays, the reconstituted stock Calibrator (10,000 pg/mL) should be store at ≤ -30°C.

2. Preparation of Rabbit Polyclonal Antibody: Reconstitute the Rabbit Polyclonal Antibody with 6 mL of Buffer Solution.

   Note: Rabbit Polyclonal Antibody must be prepared immediately before assay. Use clean test tubes or vessels.
   After reconstitution, this solution is stable for four months at 4°C.

3. Preparation of Substrate Solution: Dissolve one OPD Tablet in 12 mL of Substrate Buffer.

   Note: Substrate Solution must be prepared immediately before assay. Use clean test tubes or vessels.

4. 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.

5. Other reagents are ready for use.

STORAGE

Store kit at 4°C.

SPECIMEN COLLECTION AND HANDLING

Plasma or 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 at or below –30°C. Avoid repeated freeze/thaw cycles. EDTA-2Na additive blood collection tubes are recommended for plasma.

ASSAY PROTOCOL

1. Warm the reagents and samples to room temperature (20-30°C) before beginning the test.
2. Add 50 µL of Buffer Solution into wells. Then add 25 µL of the prepared Calibrator Solutions (0, 78.1, 156.2, 312.5, 625, 1,250, 2,500 pg/mL) or samples. Next, add 50 µL of Rabbit Polyclonal Antibody to the wells. The total volume introduced to the wells is 125 µL.
3. Cover the plate with the Plate Seal and incubate at room temperature for five hours. During incubation, the plate should be rotated on a plate rotator.
4. Remove the Plate Seal and aspirate the solution in the wells. Wash the wells three times with approximately 0.35 mL/well of Wash Solution.
5. Pipette 100 µL of HRP-Labeled Antibody into each of the wells.
6. Cover the plate with a Plate Seal and incubate at room temperature for 1 hour. During the incubation, the plate should be rotated on a plate rotator.
7. Dissolve one OPD Tablet with 12 mL of Substrate Buffer.
8. Remove the Plate Seal, aspirate and wash the wells five times with approximately 0.35 mL/well of Wash Solution.
9. Add 100 µL of Substrate Solution into the wells, cover the plate with a Plate Seal and incubate for 30 minutes at room temperature.
10. Add 100 µL of Stop Solution into the wells to stop the reaction.
11. Read the optical absorbance of the wells at 492 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.

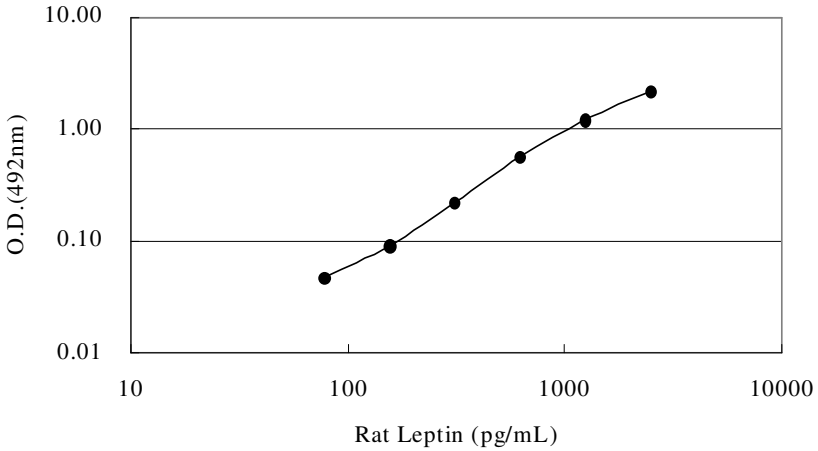
RESULTS

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 a sample value exceeds 2,500 pg/mL, it must be diluted with Buffer Solution and re-assayed until the sample value is within the assay range. If the sample value is estimated to be below 78.1 pg/mL, an additional calibrator should be prepared by diluting the 78.1 pg/mL Calibrator to 39.0 pg/mL. In this case, 40 sample in duplicate can be measured. Plot the calibrator curve using 39.0 as the lowest calibrator.

PERFORMANCE

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



Analytical Recovery

Sample	Rat Leptin Added (pg/mL)	Observed (pg/mL)	Expected (pg/mL)	Recovery (%)
Rat Serum	0.0	185.0	-	-
	62.5	248.0	247.5	100.2
	250.0	370.0	435.0	85.1
	1,000.0	920.0	1,185.0	77.7
Rat Plasma	0.0	121.0	-	-
	62.5	174.0	183.5	94.8
	250.0	292.0	371.0	78.7
	1,000.0	843.0	1,121.0	75.2

Precision and reproducibility

- Intra-assay CV (%) Serum 1.8 – 4.5
  Plasma 3.2 – 5.9
- Inter-assay CV (%) Serum 4.2 – 5.2
  Plasma 3.1 – 6.1

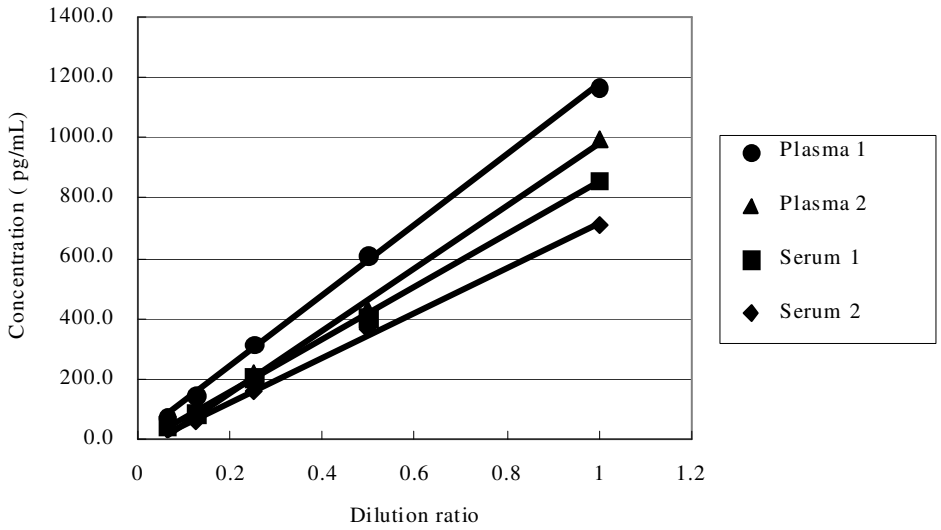
Assay Range

78.1 – 2,500 pg/mL

Cross-Reactivity

Human Leptin: 0.41%
Mouse Leptin: 0.1%
No cross-reactivity with rat IL-1α, IL-1β, rat TNF-α and other cytokines.

Dilution Test



FOR RESEARCH USE ONLY

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