KAMIYA BIOMEDICAL COMPANY

Rat Leptin ELISA

For the quantitative determination of leptin in rat plasma, serum and culture supernatant.

Cat. No. KT-437

For Research Use Only.

PRODUCT INFORMATION

Rat Leptin ELISA
Cat. No. KT-437

INTENDED USE
The Rat Leptin ELISA is for the quantitative determination of leptin in rat plasma, serum and culture supernatant. For research use only.

INTRODUCTION
Leptin, which is a product of *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 a healthy and an *ob/ob* mouse. Further, gene expression of neuropeptide Y (NPY) is surpressed by leptin. Recently, a radioimmunoassay for leptin determination in human plasma has become available and the leptin level in a human group with obesity was found to increase in comparison with that of a normal group. The level correlates well with body fat and these observations show clearly that the leptin concentration in human plasma reflects the tissue fat weight. 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 end N- and C- terminal regions between human and rat leptin. These findings have required the urgency to develop a highly sensitive immunoassay system specific to rat leptin. KAMIYA BIOMEDICAL COMPANY has developed an ELISA kit that is a stable and convenient assay system for rat leptin in plasma, serum and culture supernatant. Advantages of this kit include good quantification, no influence with other body fluid factors or physiological active substances and needlessness of sample pretreatment.

PRINCIPLE
This kit for the determination of leptin in rat plasma, serum and culture supernatant samples is based on a sandwich enzyme immunoassay. The 96-well plate is coated with anti-rat leptin monoclonal antibody. Rat leptin calibrator or samples and HRP-labeled anti-rat leptin polyclonal antibody are added to the wells for a one step sandwich immunoreaction. During this immunoreaction, monoclonal antibody – antigen – HRP-labeled antibody complex is formed. After rinsing out excess HRP-labeled antibody, HRP enzyme activity is determined 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)	Rat Leptin
3. HRP-Labeled Ab	Liquid	1 bottle (6 mL)	HRP-labeled antibody from rabbit
4. Substrate Buffer	Liquid	1 bottle (24 mL)	0.015% Hydrogen peroxide
5. OPD Tablet	Tablet	2 tablets	o-Phenylenediamine hydrochloride
6. Stop Solution	Liquid	1 bottle (12 mL)	2 N H_2SO_4
7. Buffer Solution A	Liquid	1 bottle (20 mL)	Phosphate buffer including animal serum
8. Buffer Solution B	Liquid	1 bottle (15 mL)	Phosphate buffer including surfactant
9. Wash Solution Concentrate	Liquid	1 bottle (50 mL)	Concentrated saline
10. Plate Seal		2 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 490 nm
- Rotator for microtiter plate
- Washing device for microtiter plate and dispenser for approximately 0.35 mL with aspiration system
- 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:
   (A) Sample volume 20 µL (plasma and serum):
   Reconstitute the Rat Leptin Calibrator (lyophilized, 20 ng/vial) with 1 mL of buffer solution A, which affords a 20,000 pg/mL calibrator solution. Then, 0.5 mL of the calibrator solution is diluted with 0.5 mL of buffer solution A, that yields a 10,000 pg/mL calibrator solution. Repeat the dilution to make calibrators of 5,000, 2,500, 1,250, 625 and 312.5 pg/mL. Buffer solution A is used as the 0 pg/mL calibrator.
   (B) Sample volume 50 µL (non-plasma and non-serum):
   Reconstitute the Rat Leptin Calibrator (lyophilized, 20 ng/vial) with 1 mL of buffer solution B and 0.5 mL of the reconstituted calibrator solution is diluted with 1.5 mL of buffer solution B which affords a 5,000 pg/mL calibrator solution. Then, 0.5 mL of the calibrator solution is diluted with 0.5 mL of buffer solution B, that yields a 2,500 pg/mL calibrator solution. Repeat the dilution to make calibrators of 1,250, 625, 312.5, 156.2 and 78.1 pg/mL. Buffer solution B is used as the 0 pg/mL calibrator.

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

2. Preparation of Substrate Solution: Dissolve one OPD Tablet in 11 mL of Substrate Buffer.

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

3. 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.

4. 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.

ASSAY PROTOCOL

1. Warm the reagents and samples to room temperature (20-30°C) before beginning the test.
2. Addition of calibrator solution and samples:
   (A) Sample volume 20 µL (plasma and serum):
   Add 50 µL of buffer solution A into wells first, then introduce 20 µL each of calibrator solutions (0, 312.5, 625, 1,250, 2,500, 5,000, 10,000 and 20,000 pg/mL) or samples, then add 50 µL of HRP-labeled antibody. Total 120 µL volume is introduced into the wells.
   (B) Sample volume 50 µL (non-plasma and non-serum):
   Add 50 µL each of calibrator solutions (0, 78.1, 156.2, 312.5, 625, 1,250, 2,500 and 5,000 pg/mL) or samples, then add 50 µL of HRP-labeled antibody. Total 100 µL volume is introduced into the wells.
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. During continuous rotation of test plate, the plate rotator may be heated up. It is recommended to place styrene form or plywood between the plate and the rotator. During incubation except for the color reaction, the test plate should be rotated gently by plate rotator to promote immunoreaction.
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 substrate solution into the wells, cover the plate with the Plate Seal and incubate it for 10 minutes at room temperature.
6. Add 100 µL of stop solution into the wells to stop reaction.
7. Read the optical absorbance of the wells at 490 nm. Read optical absorbance of reaction solution in wells as soon as possible after stopping the color reaction.

Note: Perform all determinations in duplicate.

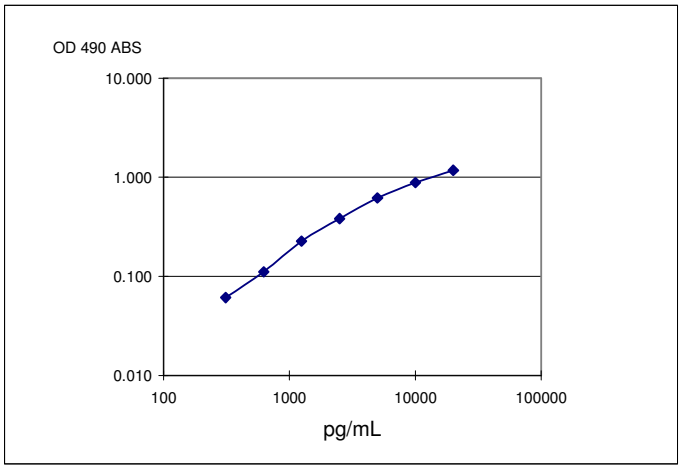
RESULTS

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

When a sample value exceeds 20,000 pg/mL, it needs to be diluted with buffer solution A until the value 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	Rat Leptin Added (pg/mL)	Observed (pg/mL)	Expected (pg/mL)	Recovery (%)
Rat Serum	0.0	0.0	-	-
	625	678	625	108.5
	2,500	2,571	2,500	102.8
	10,000	8,795	10,000	87.9
Rat Culture Supernatant	0.0	0.0	-	-
	156	169	156	108.0
	625	668	625	106.9
	2,500	2,482	2,500	99.3

Precision and reproducibility

- Intra-assay CV (%): 3.9 ~ 4.5
- Inter-assay CV (%): 6.2 ~ 9.5

Assay Range

78.1 – 5,000 pg/mL (non-plasma & non-serum sample)
312.5 – 20,000 pg/mL (plasma & serum sample)

Cross-Reactivity

The kit has high specificity to rat leptin and shows less than 0.02% – 0.04% cross-reactivity to human leptin. It also has no cross-reactivity with rat IL-1α , IL-1β , rat TNF-α , human TNF-α and other cytokines.

FOR RESEARCH USE ONLY

KAMIYA BIOMEDICAL COMPANY
12779 Gateway Drive, Seattle, WA 98168
Tel: (206) 575-8068 Fax: (206) 575-8094
Email: LifeScience@k-assay.com
www.k-assay.com