YK050　　Rat Leptin ELISA

FOR LABORATORY USE ONLY

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Contents

Ⅰ.	**Introduction**	**2**
Ⅱ.	**Characteristics**	**3**
Ⅲ.	**Composition**	**4**
Ⅳ.	**Method**	**5-6**
Ⅴ.	**Notes**	**7**
Ⅵ.	**Performance Characteristics**	**8**
Ⅶ.	**Stability and Storage**	**9**
Ⅷ.	**References**	**9**

- Please read all the package insert carefully before beginning the assay -

YK050 Rat Leptin ELISA Kit

Ⅰ. 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 suppressed by leptin.

Recently, radioimmunoassay for leptin determination in human plasma has become available and leptin level in human patient group with obesity was found to increase in comparison with that of normal group.

The level well correlate with body fat and these observations show clearly that 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 region between human and rat leptin. These findings have required urgently developing highly sensitive immunoassay system specific to rat leptin.

Yanaihara Institute Inc. has developed the enzyme immunoassay (EIA) kit, which is a stable and convenient assay system for measuring rat leptin in its plasma, serum and culture supernatant.

YK050 Rat Leptin ELISA Kit

- ▼ The assay kit can measure Leptin in the range of 312.5-20000 pg/mL (plasma or serum) and 78.1-5000 pg/mL (culture supernatant)
- ▼ The assay completes within 5.5 hours
- ▼ With one assay kit, 40 samples can be measured in duplicate
- ▼ Test sample: Plasma, serum, or culture supernatant
  Sample volume: Plasma or serum 20 µL
  Culture supernatant: 50 µL
- ▼ The 96-well plate in kit was consisted by 8-wells strips. The kit can be used separately.
- ▼ Precision and reproducibility
  Intra-assay CV (%) 3.9 - 4.5
  Inter-assay CV (%) 6.2 - 9.5
- ▼ Stability and Storage
  Store all of the components at 2-8°C.
  This kit is stable under the condition for 20 months from the date of manufacturing.
  The expiry date is described on the label of kit.

Contents

1) Antibody coated plate
2) Rat leptin standard
3) HRP-labeled antibody
4) Substrate buffer
5) OPD tablet
6) Stopping solution
7) Buffer solution A
8) Buffer solution B
9) Washing solution (concentrated)
10) Adhesive foil

Ⅱ. Characteristics

This ELISA kit is used for quantitative determination of rat leptin in its plasma, serum and culture supernatant samples. The kit is characterized for sensitive quantification, high specificity and no influences with other components in samples and needlessness of sample pretreatment. Rat leptin standard is recombinant product.

< Specificity >

This ELISA kit has high specificity to rat leptin and shows less than 0.02% - 0.04% cross reactivity to human leptin. And it has no cross reactivity with rat IL-1α, IL-1β, rat TNF-α, human TNF-α and other cytokines.

< Test Principle >

This ELISA kit for determination of rat leptin in plasma, serum and culture supernatant samples is based on a sandwich enzyme immunoassay. The 96-wells plate is coated with anti rat leptin monoclonal antibody. Rat leptin standard or samples and HRP-labeled anti rat leptin polyclonal antibody are added to the wells for one step sandwich immunoreaction. During this immunoreaction, monoclonal antibody-antigen-HRP labeled antibody complex are formed on the surface of the wells. After incubation and plate washing, HRP enzyme activity is determined by o-Phenylenediamine dihydrochloride (OPD) and the concentration of rat leptin is calculated.

Ⅲ. Composition

Component	Form	Quantity	Main Ingredient
1. Antibody coated plate	MTP*1	1 plate (96 wells)	Anti rat Leptin monoclonal antibody
2. Rat leptin standard	lyophilized	1 vial	Recombinant rat leptin (20 ng)
3. HRP-labeled antibody	liquid	1 bottle (6 mL)	HRP-labeled rabbit anti leptin antibody
4. Substrate buffer	liquid	1 bottle (24 mL)	Citrate buffer containing 0.015% Hydrogen peroxide
5. OPD tablet	tablet	2 tablets	o-Phenylenediamine dihydrochloride
6. Stopping solution	liquid	1 bottle (12 mL)	1M H_2SO_4
7. Buffer solution A	liquid	1 bottle (20 mL)	Tris-HCl buffer including serum
8. Buffer solution B	liquid	1 bottle (15 mL)	Tris-HCl buffer
9. Washing solution (Concentrated)	liquid	1 bottle (50 mL)	Concentrated saline
10. Adhesive foil		2 sheets	

MTP*1…… Microtiter plate

Ⅳ. Method

< Equipment required >

1. Photometer for microtiter plate (Plate reader), which can read extinction 2.5 at 490 nm
2. Microtiter plate shaker
3. Washing device for microtiter plate and dispenser with aspiration system
4. Micropipettes, multi-channel pipettes for 8 wells or 12 wells and their tips
5. Test tubes for preparation of standard solution
6. Graduated cylinder (1,000 mL)
7. Distilled water or deionized water

< Preparatory work >

1. Preparation of standard solution:
   (A) For plasma or serum sample (standard curve range 312.5-20,000 pg/mL, sample volume 20 μL)
   Reconstitute the Rat leptin standard (lyophilized, 20 ng/vial) with 1mL of **buffer solution A**, which affords 20,000 pg/mL standard solution. Then, the 0.2 ml of the standard solution is diluted with 0.2 mL of buffer solution A that yields 10,000 pg/mL standard solution. Repeat the dilution to make each standard solution of 5,000, 2,500, 1,250, 625, 312.5 pg/mL. Buffer solution A is used as 0 pg/mL.
   (B) For culture supernatant sample (standard curve range 78.1-5,000 pg/mL, sample volume 50 μL)
   Reconstitute the Rat Leptin standard (lyophilized, 20 ng/vial) with 1 mL of **buffer solution B** and 0.1 ml of the reconstituted standard solution is diluted with 0.3 mL of buffer solution B which affords 5,000 pg/mL standard solution. Then, 0.2 ml of the standard solution is diluted with 0.2 mL of buffer solution B that yields 2,500 pg/mL standard solution. Repeat the dilution to make each standard of 1,250, 625, 312.5, 156.2, 78.1 pg/mL. Buffer solution B is used as 0 pg/mL.

2. Preparation of substrate solution:
   Resolve one OPD tablet with 11 mL of substrate buffer. It should be prepared immediately before use.

3. Preparation of washing solution:
   Dilute 50 mL of washing solution (concentrated) to 1000 mL with distilled or deionized water.

4. Other reagents are ready for use.

< Procedure >

1. Bring all the reagents and samples return to room temperature before beginning the test.

2. Filling of standard solution and samples

(A) Sample volume 20µL (plasma and serum sample)
Fill 50µL of buffer solution A into wells first, then introduce 20 µL each of standard solutions (0, 312.5, 625, 1,250, 2,500, 5,000, 10,000, 20,000pg/mL) or samples, then add 50 µL of HRP labeled antibody. The total volume introduced into the well is 120 µL.
(B) Sample volume 50 µL (culture supernatant sample)
Fill 50µL each of standard solutions (0, 78.1, 156.2, 312.5, 625, 1,250, 2,500, 5,000 pg/mL) or samples, then add 50 µL of HRP labeled antibody. The total volume introduced into the well is 100 µL.

3. Cover the plate with adhesive foil and incubate it at room temperature (20-30°C) for 5 hours. During the incubation, the plate should be shaken with a microtiter plate shaker.

4. Take off the adhesive foil, aspirate the solution in the wells and wash the wells 5 times with approximately 0.35 mL/well of washing solution. Finally, invert the plate and tap it onto an absorbent surface, such as paper toweling, to ensure blotting free of most residual washing solution.

5. Pipette 100 µL of substrate solution into the wells, cover the plate with adhesive foil and incubate it for 10 minutes at room temperature.

6. Add 100µL of stopping solution into the wells to stop color reaction.

7. Read the optical absorbance of the wells at 490 nm. Calculate mean absorbance values of wells containing standards and plot a standard curve on logarithmic graph paper (abscissa: concentration of standard; ordinate: absorbance value). Use the standard curve to read rat leptin concentrations in samples from the corresponding absorbance values.

Ⅴ. Notes

1. Samples must be used as soon as possible after collection. If the samples are tested later, they should be divided into test tubes in small amount and frozen at or below -30°C. Avoid repeated freezing and thawing of samples.

2. Rat Leptin standard and substrate solution should be prepared immediately before use. This kit can be used dividedly in strips of the plate, in such case, the rest of reconstituted reagents (standard solution) should be stored at or below –30°C.

3. During storage of washing solution (concentrated) at 2-8°C, precipitates may be observed, however they will be dissolved when diluted.

4. Pipetting operations may affect the precision of the assay, pipette standard solutions or samples into each well of plate precisely. Using clean test tubes or vessel in assay and use a new tip for each sample to avoid cross contamination.

5. When sample (plasma, serum sample) value exceeds 20,000 pg/mL, it needs to be diluted with **buffer solution A** to a proper concentration.
   When sample (culture supernatant sample) value exceeds 5,000 pg/mL, it needs to be diluted with **buffer solution B** to a proper concentration.

6. During incubation except color reaction, the plate should be shaken gently with a microtiter plate shaker to promote immunoreaction.

7. Perform all the determination in duplicate.

8. Read optical absorbance of reaction solution in wells as soon as possible after stopping the color reaction.

9. For accurate quantification, plot a standard curve for each assay.

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

11. Satisfactory performance of the assay guaranteed only when reagents combination pack with identical lot number are used.

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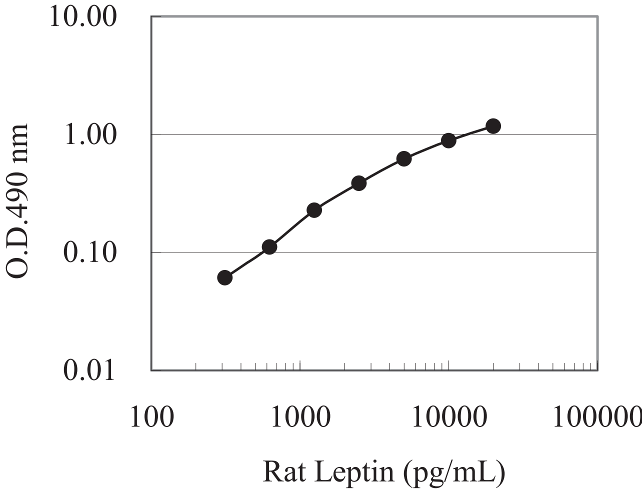
Ⅵ. Performance Characteristics

Typical standard curve (Curve range 312.5-20,000 pg/mL, Plasma or serum sample)



Analytical recovery A (Serum sample)

Rat Leptin added (pg/mL)	Observed (pg/mL)	Expected (pg/mL)	Recovery (%)
0	0	-	-
625	678	625	108.5
2500	2571	2500	102.8
10000	8795	10000	87.9

Analytical recovery B (culture supernatant sample)

Rat Leptin added (pg/mL)	Observed (pg/mL)	Expected (pg/mL)	Recovery (%)
0	0	-	-
156	169	156	108.0
625	668	625	106.9
2500	2482	2500	99.3

Precision and reproducibility

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

Assay range

312.5 - 20,000 pg/mL (plasma & serum sample)

78.1 - 5,000 pg/mL (culture supernatant sample)

Ⅶ. Stability and Storage

< Storage > Store all of the components at 2-8°C.

< Shelf life > This kit is stable under the condition for 20 months from the date of manufacturing. The expiry date is described on the label of kit.

< Package > For 96 tests per one kit including standards

Ⅷ. References

1. Zhang, Y. et al. (1994): Positional cloning of mouse obese gene and its human homologue. *Nature* ***372:*** 425-432

2. Pelleymounter, MA. et al. (1995): Effects of the obese gene product on body weight regulation in ob/ob mice. *Science* ***269*:**540-543.

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4. McGregor, GP. et al. (1996): Radiommunological measurement of leptin in plasma of obese and diabetis human subjects. *Endocrinology* ***137*:**1501-1504

5. Sainsburry, A. et al. (1996): Intracerebroventricular administration of neuropeptide Y to normal rats increase obese gene expression in white adipose tissues. *Diabetologia* ***39*:**353-356

6. Hosoda, H. et al. (1996): Development of radioimmunoassay for human leptin. *Biochem. Biophys. Res. Commun.* ***221*:**234-239