YK051　　Rat Leptin-HS ELISA

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SCETI
DF Kasumigaseki Place, 3-6-7, Kasumigaseki, Chiyoda-ku
Tokyo 100-0013 Japan
URL: http://www.sceti.co.jp/export/　e-mail: exp-pet@sceti.co.jp

Contents

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

YK051 Rat Leptin-HS 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 to develop highly sensitive immunoassay system specific to rat leptin.

Yanaihara Institute Inc. already has rat leptin ELISA kit (YK050), and now we have developed the high sensitive (HS) enzyme immunoassay kit YK051, more sensitive than YK050 which has upgrade sensitivity to 39.1 pg/mL, for measuring rat leptin in its serum and plasma.

YK051 Rat Leptin-HS ELISA Kit

▼ The assay kit can measure Leptin in the range of 78.1-2,500 pg/mL (serum or plasma)

▼ The assay completes within 6.5 hours

▼ With one assay kit, 41 samples can be measured in duplicate

▼ Test sample: Serum or plasma
Sample volume: 25 μL

▼ The 96-well plate in kit was consisted by 8-wells strips. The kit can be used separately.

▼ Precision and reproducibility

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

▼ Stability and Storage
Store all of the components at 2-8℃.
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) Specific antibody
4) HRP labeled antibody
5) Substrate buffer
6) OPD tablet
7) Stopping solution
8) Buffer solution
9) Washing solution (concentrated)
10) Adhesive foil

Ⅱ. Characteristics

This ELISA kit is used for quantitative determination of rat leptin in its serum and plasma 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 kit shows 0.41% cross reactivity to human leptin, 0.3% to mouse 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 kit for determination of rat leptin in serum and plasma samples is based on two steps sandwich enzyme immunoassay. The 96-wells plate is coated with anti rat leptin monoclonal antibody. Rat leptin standard or samples and specific antibody are added to the wells for immunoreaction. During this immunoreaction, monoclonal antibody-antigen-specific 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 specific antibody. Finally, 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.	Specific antibody	lyophilized	1 bottle	Rabbit anti rat leptin antibody
4.	HRP labeled antibody	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.	Stopping solution	liquid	1 bottle (12 mL)	1M H_2SO_4
8.	Buffer solution	liquid	1 bottle (20 mL)	Tris-HCl buffer
9.	Washing solution (Concentrated)	liquid	1bottle (50 mL)	Concentrated saline
10.	Adhesive foil		4 sheets	

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

Ⅳ. Method

< Equipment required >

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

< Preparatory work >

1. Preparation of standard solution:
   Reconstitute the Rat leptin standard (lyophilized, 20 ng/vial) with 2 mL of buffer solution that affords 10,000 pg/mL standard solution. Then, the 0.1 ml of the standard solution is diluted with 0.3 mL of buffer solution that yields 2,500 pg/mL standard solution. Then, the 0.2 ml of the 2500 pg/mL standard solution is diluted with 0.2 mL of buffer solution that yields 1,250 pg/mL standard solution. Repeat the dilution to make each standard solution of 625, 312.5, 156.2, 78.1 pg/mL. Buffer solution is used as 0 pg/mL.
2. Preparation of specific antibody
   Reconstitute specific antibody with 6 mL of buffer solution.
3. Preparation of substrate solution
   Resolve one OPD tablet with 12 mL of substrate buffer. It should be prepared immediately before use.
4. Preparation of washing solution
   Dilute 50 mL of washing solution (concentrated) to 1,000 mL with distilled or deionized water.
5. Other reagents are ready for use.

< Procedure >

1. Bring all the reagents and samples return to room temperature before beginning the test.
2. Fill 50 μL of buffer solution into wells first, then introduce 25 μL each of standard solutions (0, 78.1, 156.2, 312.5, 625, 1,250, 2,500 pg/mL) or samples, then add 50 μL of specific antibody. The total volume introduced into the well is 125 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 3 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 HRP labeled antibody into the wells
6. Cover the plate with adhesive foil and incubate it for 1 hour at room temperature. During the incubation, the plate should be shaken with a microtiter plate shaker.
7. Resolve one OPD tablet with 12 mL of substrate buffer. It should be prepared immediately before use.
8. 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.
9. Pipette 100 μL of substrate solution into the wells, cover the plate with adhesive foil and incubate it for 30 minutes at room temperature.
10. Add 100 μL of stopping solution into the wells to stop color reaction.
11. 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

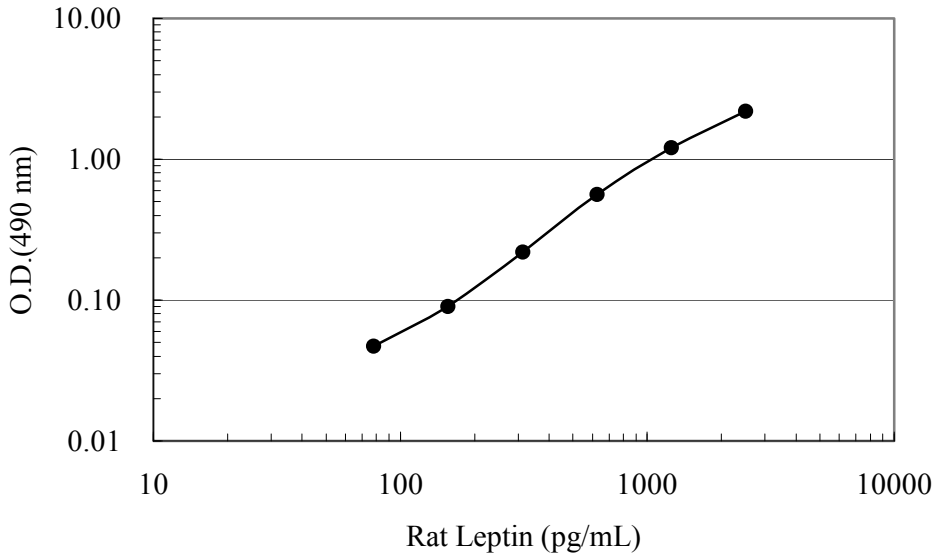
1. Samples must be used as soon as possible after collection. If the samples are tested later, they should be divided into test tubes in small amount and frozen at or below -30°C. Avoid repeated freezing and thawing of samples. EDTA-2Na additive blood collection tube is recommended for the plasma.
2. Rat leptin standard, specific antibody and substrate solution should be prepared immediately before use. This kit can be used dividedly in strips of the plate, in such case, the standard solution (10,000 pg/mL) and the specific antibody should be stored at or below than -30°C, but specific antibody is stable for 4 months at 4°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 precision of the assay, pipette standard solutions or samples precisely into each well of plate. Using clean test tubes or vessels in assay and use a new tip for each sample and standard to avoid cross contamination.
5. When sample value exceeds 2,500 ng/mL, it needs to be diluted with buffer solution to a proper concentration.
6. During the incubation at room temperature except color reaction, the test plate should be shaken gently with a microtiter plate shaker to promote immunoreaction.
7. Perform all the determination in duplicate.
8. To quantitate accurately always run a standard curve when testing samples.
9. Read optical absorbance of reaction solution in wells as soon as possible after stopping the color reaction.
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.

Ⅵ. Performance Characteristics

<Assay range> 78.1～2,500 pg/mL (plasma or serum)

If the sample value estimates below the 78.1 pg/mL, it should be diluted 78.1 pg/mL standard solution to 39.0 pg/mL, namely one more standard solution should be set up. In this case, 40 samples can be measured in duplicate. Use the calculated sample value which is between the concentration of 39.0pg/mL～78.1 pg/mL as an approximate value.

Typical standard curve



<Analytical recovery>

Serum sample

Sample No.	Leptin added (pg/mL)	Observed (pg/mL)	Expected (pg/mL)	Recovery (%)
1	0.0	185.0	-	-
2	62.5	248.0	247.5	100.2
3	250.0	370.0	435.0	85.1
4	1000.0	920.0	1185.0	77.7

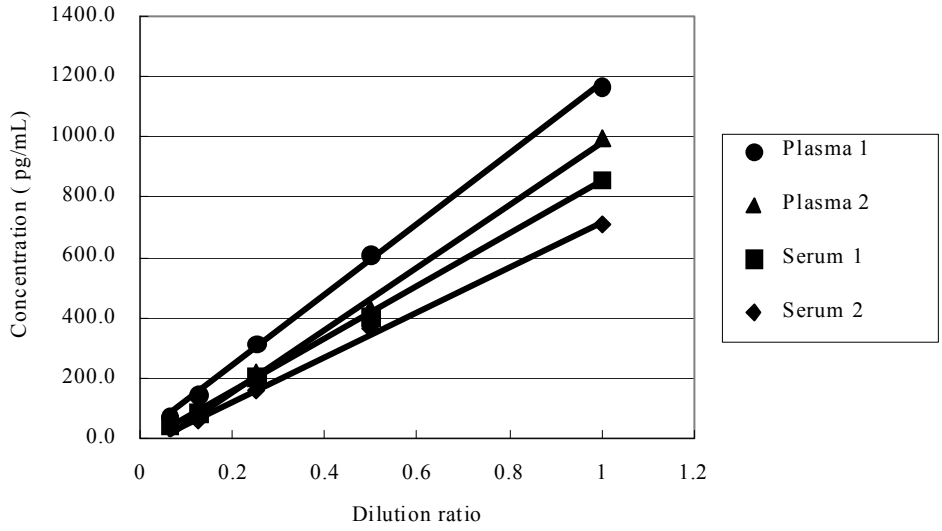
Plasma sample

Sample No.	Leptin added (pg/mL)	Observed (pg/mL)	Expected (pg/mL)	Recovery (%)
1	0.0	121.0	-	-
2	62.5	174.0	183.5	94.8
3	250.0	292.0	371.0	78.7
4	1000.0	843.0	1121.0	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

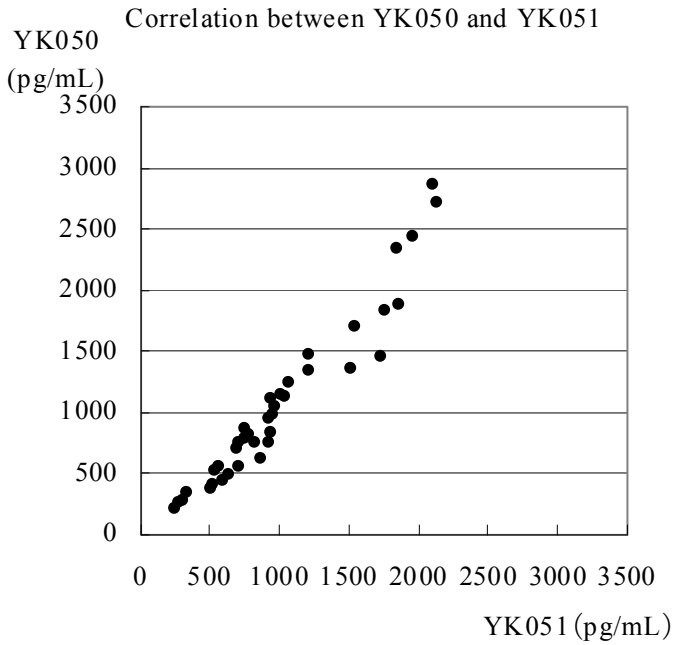
＜Dilution test＞



＜Correlation between YK050 and YK051＞

Y＝YK050 Rat Leptin ELISA,　X=YK051 Rat Leptin-HS ELISA

Y = 1.26X - 179　r = 0.968　n = 38



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

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5. Sainsburry, A. et al. (1996): Intracerebroventricular administration of neuropeptide Y to normal rats increases obese gene expression in white adipose tissues. *Diabetologia* ***3,*** 353-356
6. Hosoda, H. et al. (1996): Development of radioimmunoassay for human leptin. *Biochem. Biophys. Res. Commun.* ***221,*** 234-239

<Manufacturer>

Yanaihara Institute Inc.
2480-1 Awakura, Fujinomiya-shi
Shizuoka, Japan 418-0011
TEL: +81-544-22-2771 FAX: +81-544-22-2770
Website: http://www.yanaihara.co.jp E-mail: ask@yanaihara.co.jp

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