YK052 Mouse Leptin ELISA

FOR LABORATORY USE ONLY

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Contents

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

YK052 Mouse Leptin ELISA Kit

Ⅰ. Introduction

Leptin, which is a product of *ob* gene, is a protein consisting of 146 amino acids and it is secreted from white adipose tissue. It is known that leptin acts on hypothalamus to decrease food intake and reduce body weight, body fat, blood sugar and blood insulin in a healthy and an *ob/ob* mice. Further, gene expression of neuropeptide Y (NPY) is suppressed by this protein. 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 in normal group. The plasma level well correlated with body fat and these observations showed clearly that leptin concentration in human plasma reflects tissue fat weight. The measurement of leptin in plasma or serum may be a good index of obesity. Although mouse leptin shows a high homology (96%) with rat leptin in amino acid sequences, it is observed that substitution of quite a number of residues occurs between human and mouse leptin. These findings have urged us to develop highly sensitive immunoassay system specific to rat or mouse leptin.
Yanaihara Institute Inc. already has rat leptin ELISA kit (YK050 and YK051), and now we have developed mouse leptin ELISA kit for measuring mouse leptin in its serum. It will be a specifically useful tool for leptin researches.

YK052 Mouse Leptin ELISA Kit

- ▼ The assay kit can measure mouse leptin within the range of 0.313-20 ng/mL.
- ▼ The assay is completed within 6.5 hr.
- ▼ With one assay kit, 40 samples can be measured in duplicate.
- ▼ Test sample: Mouse serum
  Sample volume: 25 μL
- ▼ The 96-wells plate in kit is consisted by 8-wells strips, and the strips can be used separately.
- ▼ Precision and reproducibility
  Intra-assay CV（%） Mouse serum 5.01-9.84
  Inter-assay CV（%） Mouse serum 4.37-7.71
- ▼ Stability and storage
  Store all of the components at 2-8℃.
  The kit is stable under the condition for 24 months from the date of manufacturing.
  The expiry date is stated on the label of kit.

Contents

1) Antibody coated plate
2) Standard
3) Labeled antibody solution
4) SA-HRP solution
5) Enzyme substrate solution (TMB)
6) Stopping solution
7) Buffer solution
8) Washing solution (concentrated)
9) Adhesive foil

Ⅱ. Characteristics

This ELISA kit is used for quantitative determination of mouse leptin in its serum samples. The kit is characterized by its sensitive quantification and high specificity. In addition, it is not influenced by other constituents in samples. Standard antigen, mouse leptin in this kit is a recombinant product.

< Specificity >
This ELISA kit is highly specific to mouse leptin. It shows 33.8 % crossreactivity to rat leptin and no crossreactivity to human leptin.

< Assay principle >
This ELISA kit for determination of mouse leptin is based on a sandwich enzyme immunoassay. To the wells of plate coated with highly purified antibody against mouse leptin, standard antigen or sample is added for the 1st step immunoreaction. After the 1st step incubation and plate washing, biotinylated rabbit anti mouse leptin antibody is added as the 2nd step reaction to form leptin antibody- antigen -biotinylated leptin antibody complex on the surface of the wells. After the 2nd step incubation and rinsing out excess biotinylated antibody, horse radish peroxidase (HRP) labeled streptoavidin (SA) is added for binding to biotinylated leptin antibody. Finally, HRP enzyme activity is determined by 3,3',5,5'-Tetramethylbenzidine (TMB) and the concentration of mouse leptin is calculated.

Ⅲ. Composition

	Component	Form	Quantity	Main Ingredient
1.	Antibody coated plate	microtiter plate	1 plate (96 wells)	Rabbit anti mouse leptin antibody
2.	Standard	lyophilized	1 vial (20ng)	Recombinant mouse leptin
3.	Labeled antibody solution	liquid	1 bottle (12 mL)	Biotinylated rabbit anti mouse leptin antibody
4.	SA-HRP solution	liquid	1 bottle (12 mL)	Horseradish peroxidase labeled streptoavidin
5.	Enzyme substrate solution	liquid	1 bottle (12 mL)	3,3',5,5'-Tetramethylbenzidine (TMB)
6.	Stopping solution	liquid	1 bottle (12 mL)	1M H_2SO_4
7.	Buffer solution	liquid	1 bottle (15 mL)	Phosphate buffer
8.	Washing solution (concentrated)	liquid	1 bottle (50 mL)	Concentrated saline
9.	Adhesive foil		4 pieces	

Ⅳ. Method

< Equipment required >

1. Photometer for microtiter plate (plate reader), which can read extinction 2.5 at 450nm.
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. Glass test tubes for preparation of standard solution
6. Graduated cylinder (1,000 mL)
7. Distilled or deionized water

< Preparatory work >

1. Preparation of standard solution:
   Reconstitute the mouse leptin standard with 1 mL of buffer solution, which affords 20 ng/mL standard solution. The reconstituted standard solution (0.2 mL) is diluted with 0.2mL of buffer solution that yields 10 ng/mL standard solution. Repeat the dilution procedure to make each standard solution of 5, 2.5, 1.25, 0.625 and 0.313 ng/mL. Buffer solution itself is used as 0 ng/mL standard solution.
   If a sample concentration below 0.313 ng/mL is predicted, standard curve may be further set up a lower detection limit by using 0.156 ng/mL standard solution which can be prepared by 2-fold dilution of 0.313 ng/mL standard solution. In such case, however, assay precision may not be so excellent as that of the cases between 0.313 and 20 ng/mL.

2. Preparation of washing solution:
   Dilute 50 mL of washing solution (concentrated) to 1,000 mL with distilled or deionized water.

3. Other reagents are ready for use.

< Procedure >

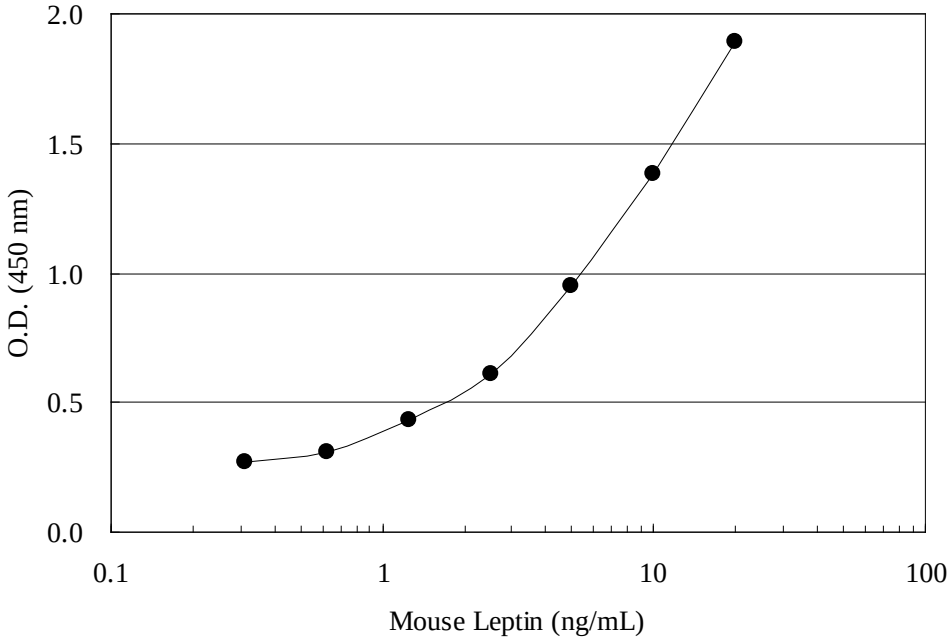
1. Bring all the reagents and samples to room temperature (20-30°C) at least 1 hour before starting assay.
2. Add 0.35mL/well of washing solution into the wells of the plate, and then aspirate the solution. Repeat this washing procedure further twice (total 3 times). Finally, invert the plate and tap it onto an absorbent surface, such as paper toweling, to ensure blotting free of most residual washing solution.
3. Fill 45µL of buffer solution into each of wells first, and then add 25µL each of standard 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 adhesive sheet and incubate it at room temperature for 3 hours. During incubation, the plate should be shaken with a microtiter plate shaker.
5. After incubation, take off the adhesive sheet, aspirate the solution in the wells and wash the wells 4 times with approximately 0.35 mL/well each 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.
6. Pipette 100µL of labeled antibody solution into each of the wells.
7. Cover the plate with adhesive sheet and incubate it at room temperature for 2 hours. During incubation, the plate should be shaken with a microtiter plate shaker.
8. Take off the adhesive sheet, aspirate the solution in the wells and then wash the wells 4 times with approximately 0.35 mL/well each 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 100µL of SA-HRP solution into each of the wells.
10. Cover the plate with adhesive sheet and incubate it at room temperature for 1 hour. During incubation, the plate should be shaken with a microtiter plate shaker.
11. Pipetting the required volume of enzyme substrate (TMB) solution into a vessel, and bring it to room temperature (20-30°C) under a light-proof condition for 1 hour before use. Store the rest of the TMB solution at 2-8℃ for next use.
12. Take off the adhesive sheet, aspirate the solution in the wells and then wash the wells 4 times with approximately 0.35 mL/well each 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.
13. Add 100 µL of TMB solution into each of the wells, cover the plate with adhesive sheet and keep it for 30 minutes at room temperature under a light-proof condition (keep still, plate shaker not need).
14. Add 100 µL of reaction stopping solution into each of the wells.
15. Read the optical absorbance of the solution in the wells at 450 nm. The dose-response curve of this assay fits best to a 4 (or 5)-parameter logistic equation. The results of unknown samples can be calculated with any computer program having a 4 (or 5)-parameter logistic function.
    Otherwise calculate mean absorbance values of wells containing standards and plot a standard curve on semilogarithmic graph paper (abscissa: concentration of standard; ordinate: absorbance values). Use the average absorbance of each sample to determine the corresponding value by simple interpolation from this standard curve.

Ⅴ. Notes

1. It is strongly recommended that serum samples should 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 below -30°C. Avoid repeated freezing and thawing of samples.
2. Standard antigen solution should be prepared immediately before use. This kit can be used dividedly in strips of the plate. In such a case, the rest of reconstituted reagents including standard solutions should be stored below -30°C（stable for 1 month）.
3. During storage of washing solution (concentrated) at 2-8°C, precipitates may be observed. However, they will be dissolved when diluted. Diluted washing solution is stable for 6 months at 2-8°C.
4. Pipetting operations may affect precision of the assay. Pipette standard solutions or samples into each well of the plate precisely. Use clean test tubes and vessels in assay, and new tip must be used for each sample and standard solution to avoid cross contamination.
5. When concentration of leptin in sample is expected to exceed 20 ng/mL, the sample needs to be diluted with buffer solution to a proper concentration.
6. During incubation, except the case of 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 the wells immediately after stopping color reaction.
9. For accurate quantification, plot a standard curve for each assay.
10. Protect reagents from strong light (e.g. direct sunlight) during assay and storage.
11. Satisfactory performance of assay is guaranteed only when reagents in combination pack with identical lot number are used.
12. Pipetting the required volume of enzyme substrate (TMB) solution into a vessel, and bring it to room temperature (20-30°C) under a light-proof condition 1 hour before use. Color reaction by TMB must be carried on under a light-proof condition.
13. Some reagents contain human serum (tested and found negative for HBsAG, HIV 1/2, HCV, HIV-1 AG or HIV-1 NAT, ALT and a test for Syphilis by FDA approved methods), care should be taken when handling.

Ⅵ. Performance Characteristics

A typical standard curve



<Analytical recovery>

＜Mouse serum 1＞

Added leptin (ng/ml)	Observed (ng/ml)	Expected (ng/ml)	Recovery (%)
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＞

Added leptin (ng/ml)	Observed (ng/ml)	Expected (ng/ml)	Recovery (%)
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＞

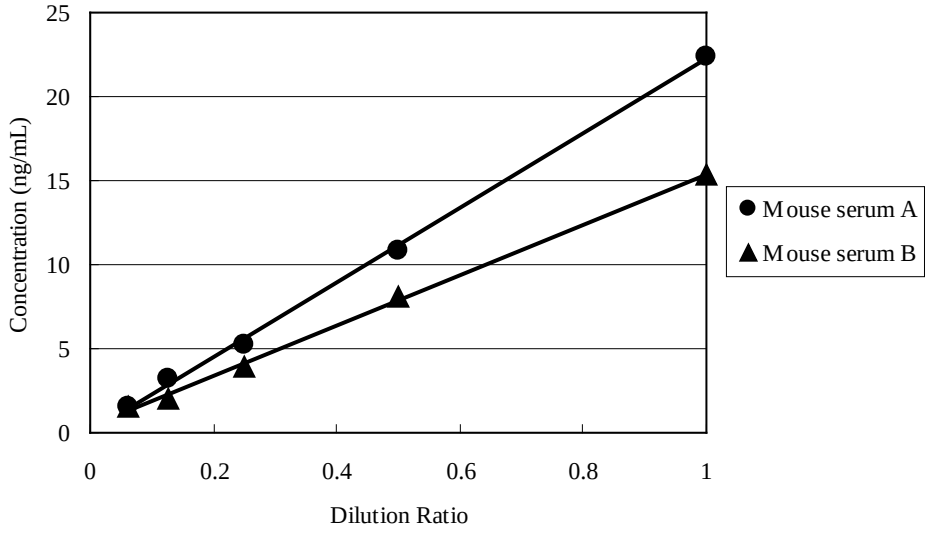
Added leptin (ng/ml)	Observed (ng/ml)	Expected (ng/ml)	Recovery (%)
0.0	1.20		
0.3	1.58	1.50	105.33
3.0	3.90	4.20	92.86
7.0	8.91	8.20	108.66

＜Mouse serum 4＞

Added leptin (ng/ml)	Observed (ng/ml)	Expected (ng/ml)	Recovery (%)
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

<Dilution test>

Mouse serum



＜Crossreactivity＞

Related protein	Crossreactivity（%）
Mouse leptin	100.0
Rat leptin	33.8
Human leptin	0.0

< Precision and reproducibility >

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

Ⅶ. Stability and Storage

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

< Shelf life > The kit is stable under the condition for 24 months from the date of manufacturing. The expiry date is stated on the package.

< Package > For 96 tests per one kit.

Ⅷ. References

1. Zhang, Y et al: Positional cloning of the mouse obese gene and its human homologue. Nature 372, 425, 1994
2. Pelleymounter, MA et al: Effects of the obese gene and product on body weight regulation in ob/ob mice. Science 299, 540, 1995
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4. McGregor, G et al: Radioimmunological measurement of leptin in plasma of obese and diabetis human subject. Endocrinology 137, 1501, 1996
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7. Hashimoto, H et al: Parathyroid hormone-related protein induces cachectic syndromes without directly modulating the expression of hypothalamic feeding-regulating peptides. Clin Cancer Res 13, 292, 2007

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