Code No. 27295

Rat Leptin Assay Kit - IBL

INTRODUCTION

The recessive obesity mutation, ob, identified in 1950, results in profound obesity and type II diabetes as part of a syndrome that resembles morbid obesity in humans. Freidman's group at Rockefeller University originally identified the obesity gene that encodes for Leptin in 1994. Leptin is a 16 kDa secreted protein produced by the ob gene. Adipose tissue produces Leptin and releases it into the bloodstream. As fat deposits grow, blood Leptin levels tends to increase. It has been suggested that Leptin acts as a lipostat, increasing as fat gets deposited into adipocytes. It is also found that the protein acts as a hormone instructing the brain to stop food consumption and to increase activity. The protein has also been shown to signal and probably control the onset of puberty. In addition, recent study shows Leptin acts as a potent inhibitor of bone formation through central nervous system.

PRINCIPLE

This kit is a solid phase sandwich ELISA using 2 kinds of high specific antibodies. Tetra Methyl Benzidine (TMB) is used as coloring agent (Chromogen). The strength of coloring is in proportion to the quantities of Rat Leptin.

MEASUREMENT RANGE

56.25 ~ 3,600 pg/mL

INTENDED USE

For research use only, not for use in diagnostic procedures.

The IBL's Rat Leptin EIA Kit is a complete kit for the quantitative determination of Rat Leptin in serum, EDTA-plasma and supernatant of cell culture media.

KIT COMPONENT

1	Precoated plate	: Anti- Rat Leptin Rabbit IgG Affinity Purify	96Well x 1
2	Labeled antibody Conc.	: (30X) HRP conjugated Anti- Rat Leptin Rabbit IgG Fab' Affinity Purify	0.4mL x 1
3	Standard	: Native Rat Leptin	0.5mL x 2
4	EIA buffer*		30mL x 1
5	Solution for Labeled antibody*		12mL x 1
6	Chromogen	: TMB solution	15mL x 1
7	Stop solution*		12mL x 1
8	Wash buffer Conc.*		50mL x 1

OPERATION MANUAL

1. Materials needed but not supplied

- Plate reader (450nm)
- Graduated cylinder and beaker
- Incubator (37℃±1℃)
- Paper towel
- Plate washer or washing bottle*
- Disposable test tube for "2, Labeled antibody Conc." and "6, Chromogen"
- Micropipette and tip
- Distilled water
- Graph paper (log/log)
- Tube for dilution of Standard

2. Preparation

1) Preparation of wash buffer
"8, Wash buffer Conc." is a concentrated (X40) buffer. The temperature of "8, Wash buffer Conc." shall be adjusted to room temperature and then, mix it gently and completely before use. Dilute 50mL of "8, Wash buffer Conc." with 1,950mL of distilled water and mix it. This is the wash buffer for use. This prepared wash buffer shall be stored in refrigerator and used within 2 weeks after dilution.

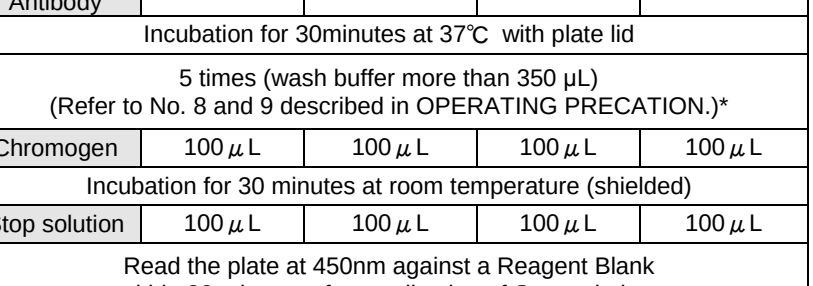
2) Preparation of Labeled antibody
"2, Labeled antibody Conc." is a concentrated (X30). Dilute "2, Labeled antibody Conc." with "5, Solution for Labeled antibody" in 30 times according to required quantity into a disposable test tube. Use this resulting solution as Labeled antibody.
Example)
In case you use one slit (8 well), the required quantity of Labeled antibody is 800 μL. (Dilute 30 μL of "2, Labeled antibody Conc." with 870 μL of "5, Solution for Labeled antibody" and mix it. And use the resulting solution by 100 μL in each well.)
<u>This operation should be done just before the application of Labeled antibody.</u>
The remaining "2, Labeled antibody Conc." should be stored at 4℃ in firmly sealed vial.

3) Preparation of Standard
Put just 0.5mL of distilled water into the vial of "3, Standard" and mix it gently and completely. This solution is 7,200pg/mL Rat Leptin standard.

4) Dilution of Standard
Prepare 8 tubes for dilution of "3, Standard". Put 230 μL each of "4, EIA buffer" into the tube.
Specify the following concentration of each tube.

Tube-1	3,600 pg/mL
Tube-2	1,800 pg/mL
Tube-3	900 pg/mL
Tube-4	450 pg/mL
Tube-5	225 pg/mL
Tube-6	112.5 pg/mL
Tube-7	56.25 pg/mL
Tube-8	0 pg/mL (Test Sample Blank)

Put 230 μL of Standard solution into tube–1 and mix it gently. Then, put 230 μL of tube-1mixture into tube-2. Dilute two times standard solution in series to set up 7 points of diluted standard between 3,600pg/mL and 56.25pg/mL. Tube-8 is the test sample blank as 0pg/mL.
See following picture.



5) Dilution of test sample
Test sample should be diluted with "4, EIA buffer" as the need arises.
Example: rat serum (X10)
If the concentration of Rat Leptin in samples may not be estimated in advance, the pre-assay with several different dilutions will be recommended to determine the proper dilution of samples.

3. Measurement procedure

All reagents shall be brought to room temperature approximately 30 minutes before use. Then mix it gently and completely before use. Confirm no change in quality of the reagents. Standard curve shall be prepared simultaneously with the measurement of test samples.

Reagents	Test Sample	Standard	Test Sample Blank	Reagent Blank
	Test sample 100 μL	Diluted standard (Tube 1~7) 100 μL	EIA buffer (Tube-8) 100 μL	EIA buffer 100 μL
Incubation for 1 hour at 37℃ with plate lid				
4 times (wash buffer more than 350 μL) (Refer to No. 8 and 9 described in OPERATING PRECATION.)*				
Labeled Antibody	100 μL	100 μL	100 μL	-
Incubation for 30minutes at 37℃ with plate lid				
5 times (wash buffer more than 350 μL) (Refer to No. 8 and 9 described in OPERATING PRECATION.)*				
Chromogen	100 μL	100 μL	100 μL	100 μL
Incubation for 30 minutes at room temperature (shielded)				
Stop solution	100 μL	100 μL	100 μL	100 μL
Read the plate at 450nm against a Reagent Blank within 30 minutes after application of Stop solution.				

1) Determine wells for reagent blank. Put 100 μL each of "4, EIA buffer" into the wells.
2) Determine wells for test sample blank, test sample and diluted standard. Then, put 100 μL each of test sample blank (tube-8), test sample and dilutions of standard (tube-1~7) into the appropriate wells.
3) Incubate the precoated plate for 1 hour at 37℃ after covering it with plate lid.
4) Washing (Refer to No. 8 and 9 described in OPERATING PRECATION.)*
5) Pipette 100 μL of labeled antibody solution into the wells of test samples, diluted standard and test sample blank.
6) Incubate the precoated plate for 30 minutes at 37℃ after covering it with plate lid.
7) Washing (Refer to No. 8 and 9 described in OPERATING PRECATION.)*
8) "6, Chromogen" should be taken the required quantity into a disposable test tube. Then, pipette 100 μL from the test tube into the wells. Please avoid to return the rest of test tube into "6, Chromogen" bottle due to avoid to cause of contamination.
9) Incubate the precoated plate for 30 minutes at room temperature in the dark. The liquid will turn blue by the addition of "6, Chromogen".
10) Pipette 100 μL of "7, Stop solution" into the wells. Mix the liquid by tapping the side of precoated plate. The liquid will turn yellow by the addition of "7, Stop solution".
11) Remove any dirt or drop of water on the bottom of the precoated plate and confirm there is no bubble on the surface of the liquid. Then, run the plate reader and conduct measurement at 450nm.
The measurement shall be done within 30minutes after the addition of "7, Stop solution".

OPERATING PRECATION*

1) Test samples should be measured soon after collection. For storage of samples, store them frozen and do not repeat freeze/thaw cycles. Thaw the test samples at a low temperature and mix them completely before measurement.
2) Test samples should be diluted with "4, EIA buffer" contained in this kit.
3) Duplicate measurement of test samples and standards is recommended.
4) Standard curve should run for each assay.

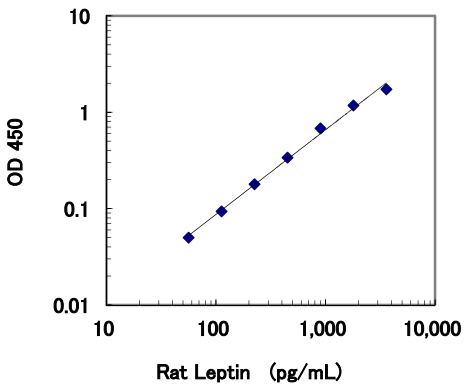
5) Use test samples in neutral pH range. The contaminations of organic solvent may affect the measurement.
6) All reagents should be brought to room temperature (R.T.) and mixed completely and gently before use. After mixing them, make sure of no change in quality of the reagents.
7) Use only “8, Wash buffer conc.” contained in this kit for washing the precoated plate. Insufficient washing may lead to the failure in measurement.
8) Using a plate washer is recommended (wait time zero second). It should be washed by a plate washer immediately after each reaction. If you use a washing bottle instead of a plate washer, after filling wash buffer in each well, immediately turn the plate upside down and shake it off to completely remove the wash buffer. Repeat the number of times of wash defined in a table for measurement procedure described in section 3. It should be properly washed off as instructed in order to avoid any insufficient wash.
9) Carefully tap the plate against a clean paper towel without contacting with inside of each well to completely remove the washing buffer after repeated the determined number of wash.
10) “6, Chromogen - TMB solution” should be stored in the dark due to its sensitivity against light. It should be also avoided contact with metals. Required quantity should be prepared into a collecting container for each use.
11) After adding TMB solution into the wells, the liquid in the wells gradually changes the color in blue. In this process the plate should be in dark. Remained TMB solution in the collecting container should not be returned into the original bottle of TMB solution to avoid contamination.
12) Measurement of O.D. should be done within 30 minutes after addition of “7, Stop solution”.

CALCULATION OF TEST RESULT

Subtract the absorbance of test sample blank from all data, including standards and unknown samples before plotting. Plot the subtracted absorbance of the standards against the standard concentration on log-log graph paper. Draw the best smooth curve through these points to construct the standard curve. Read the concentration for unknown samples from the standard curve.

Example of standard curve

Conc. (pg/mL)	Absorbance (450nm)
3,600	1.805
1,800	1.252
900	0.755
450	0.414
225	0.255
112.5	0.169
56.25	0.126
0 (Test Sample Blank)	0.076



＊ The typical standard curve is shown above. This curve can not be used to derive test results. Please run a standard curve for each assay.

PERFORMANCE CHARACTERISTIC

1. Titer Assay (Samples with standard added are used.)

Specimen	Titer (X)	Measurement Value (pg/mL)	Theoretical Value (pg/mL)	%
10% FCS added RPMI-1640	2	861.42	900.00	95.7
	4	458.03	450.00	101.8
	8	217.74	225.00	96.8
	16	104.87	112.50	93.2
Rat Serum	2	858.71	1,088.44	78.9
	4	490.89	551.50	89.0
	8	262.04	276.12	94.9
	16	135.13	138.85	97.3
Rat Plasma (EDTA) (Wistar)	8	3,129.75	3,203.63	97.7
	16	1,624.82	1,646.38	98.7
	32	857.96	886.57	96.8
Rat Plasma (EDTA) (Wistar) (x20)	1,953.47	1,916.63	98.1	
	1,503.47	1,465.22	97.5	
	1,278.47	1,249.18	97.7	

2. Added Recovery Assay

Specimen	Theoretical Value (pg/mL)	Measurement Value (pg/mL)	%
10% FCS added RPMI-1640 (x2)	900.00	841.20	93.5
	450.00	419.72	93.3
	225.00	196.42	87.3
Rat Serum (x8)	942.58	860.64	91.3
	492.58	471.57	95.7
	267.58	241.57	90.3

3. Intra – Assay

Measurement Value (pg/mL)	SD value	CV value (%)	n
2,377.16	74.89	3.2	24
570.04	18.87	3.3	24
130.18	5.74	4.4	24

4. Inter – Assay

Measurement Value (pg/mL)	SD value	CV value (%)	n
2,432.58	123.69	5.1	32
577.85	23.43	4.1	32
130.63	6.09	4.7	32

5. Specificity

Compound	Cross Reactivity
Rat Leptin	100.0%
Mouse Leptin	17.9%
Human Leptin	0.2%

6. Sensitivity

10.82 pg/mL
The sensitivity for this kit was determined using the guidelines under the National Committee for Clinical Laboratory Standards (NCCLS) Evaluation Protocols. (National Committee for Clinical Laboratory Standards Evaluation Protocols, SC1, (1989) Villanova, PA: NCCLS.

PRECAUTION FOR INTENDED USE AND/OR HANDLING

1. All reagents should be stored at 2~8℃. All reagents shall be brought to room temperature approximately 30 minutes before use.
2. “3, Standard” is lyophilized products. Be careful to open this vial.
3. “7, Stop solution” is a strong acid substance. Therefore, be careful not to contact your skin and clothes with “7, Stop solution” and pay attention to the disposal of “7, Stop solution”.
4. Dispose used materials after rinsing them with large quantity of water.
5. The precipitation may grow in “2, Labeled antibody Conc.”, however, there is no problem in the performance.
6. Wash hands after handling reagents.
7. Do not mix the reagents with the reagents from different lot or different kit.
8. Do not use the reagents expired.
9. This kit is for research purpose only. Do not use for clinical diagnosis.

STORAGE AND THE TERM OF VALIDITY

Storage Condition : 2 - 8 °C
The expiry date is specified on outer box.

REFERENCES

1. Masaki T. et al. Enhanced expression of uncoupling protein 2 gene in rat white adipose tissue and skeletal muscle following chronic treatment with thyroid hormone. *FEBS letters.* 1997: 418 (3), 323 - 326.
2. Masaki, T. et al. Induction of rat uncoupling protein-2 gene treated with tumor necrosis factor alpha in vivo. *Euro. J. Clin. Invest.* 1999: 29 (1), 76 - 82.
3. Masaki T. et al. Tumor necrosis factor-alpha regulates in vivo expression of the rat UCP family differentially. *Biochim. Biophys. Acta.* 1999: 143 (3), 585 - 592.
4. Ducy P et al. Leptin inhibits bone formation through a hypothalamic relay: A central control of bone mass. *Cell* 2000: 100 (2), 197-207

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Made in Japan.