LEPR (Human) ELISA Kit

Catalog Number KA0027

96 assays

Version: 07

Intended for research use only

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Introduction

Intended Use

The LEPR (Human) ELISA Kit is a sandwich enzyme immunoassay for the quantitative measurement of human leptin receptor.

Features

- ✓ It is intended for research use only.
- ✓ The total assay time is less than 2.5 hours.
- ✓ The kit measures leptin receptor in serum, plasma (EDTA and heparin).
- ✓ Assay format is 96 wells.
- ✓ Quality Controls are human serum based.
- ✓ Standard is recombinant protein based.
- ✓ Components of the kit are provided ready to use, concentrated or lyophilized.

Background

Leptin receptor (OB-R) was identified as a leptin binding protein (Leptin, the product of the *ob* gene, is a single-chain 16 kDa protein consisting of 146 amino acid residues.) OB-R was found to be a member of the class I cytokine receptor family with a large extracellular domain. Leptin receptor exists in multiple forms with a common extracellular domain and a variable length cytoplasmatic portion. Alternate splicing from a single gene derives the six isoforms of the leptin receptor.
The soluble form of the leptin receptor, OB-R contains no intracellular motifs or transmembrane residues, thus it consists entirely of the extracellular ligand-binding domain of the receptor.
Long forms of OB-R transcripts were reported to be expressed predominantly in regions of the hypothalamus which provides evidence that leptin receptor is important in body weight regulation. Expression of short forms of OB-R transcripts have been found in multiple tissues, including the choroid plexus, lung, kidney, and primitive hematopoietic cell populations. Leptin receptor may act as a negative regulator of leptin activity and it may maintain a pool of available bioactive leptin by binding and delaying its clearance from circulation.
Soluble leptin receptor levels are indirectly proportional to adiposity and are increased in females versus males. Leptin receptor levels are highest in infants, decrease into adolescence, and remain relatively stable throughout adulthood. Soluble leptin receptor is also found upregulated in patients with chronic heart failure, end-stage renal disease and anorexia.

Areas of investigation:
Energy metabolism and body weight regulation

Principle of the Assay

In the LEPR (Human) ELISA Kit, Standards, Quality Controls and samples are incubated in microplate wells pre-coated with monoclonal anti-human leptin receptor antibody. After 60 minutes incubation and washing, monoclonal anti-human leptin receptor antibody, conjugated with horseradish peroxidase (HRP) is added to the wells and incubated for 60 minutes with captured leptin receptor. Following another washing step, the remaining HRP conjugate is allowed to react with the substrate solution (TMB). The reaction is stopped by addition of acidic solution and absorbance of the resulting yellow product is measured. The absorbance is proportional to the concentration of leptin receptor. A standard curve is constructed by plotting absorbance values against concentrations of Standards, and concentrations of unknown samples are determined using this standard curve.

General Information

Materials Supplied

List of component

Component	State	Amount
Antibody Coated Microtiter Strips	ready to use	96 wells
Conjugate Solution	ready to use	13 mL
Master Standard	lyophilized	2 vials
Quality Control HIGH	lyophilized	1 vial
Quality Control LOW	lyophilized	1 vial
Dilution Buffer	ready to use	13 mL
Wash Solution Conc. (10x)	concentrated	100 mL
Substrate Solution	ready to use	13 mL
Stop Solution	ready to use	13 mL

Storage Instruction

Store the complete kit at 2-8°C. Under these conditions, the kit is stable until the expiration date (see label on the box).

Materials Required but Not Supplied

- ✓ Deionized (distilled) water
- ✓ Test tubes for diluting samples
- ✓ Glassware (graduated cylinder and bottle) for Wash Solution (Dilution Buffer)
- ✓ Precision pipettes to deliver 10-1000 µL with disposable tips
- ✓ Multichannel pipette to deliver 100 µL with disposable tips
- ✓ Absorbent material (e.g. paper towels) for blotting the microtitrate plate after washing
- ✓ Vortex mixer
- ✓ Orbital microplate shaker capable of approximately 300 rpm
- ✓ Microplate washer (optional). [Manual washing is possible but not preferable.]
- ✓ Microplate reader with 450 ± 10 nm filter, preferably with reference wavelength 630 nm (alternatively another one from the interval 550 - 650 nm)
- ✓ Software package facilitating data generation and analysis (optional)

Precautions for Use

- Precautions

✓ For professional use only.

✓ Wear gloves and laboratory coats when handling immunodiagnostic materials.

✓ Do not drink, eat or smoke in the areas where immunodiagnostic materials are being handled.

✓ This kit contains components of human origin. These materials were found non-reactive for HBsAg, HCV antibody and for HIV 1/2 antigen and antibody. However, these materials should be handled as potentially infectious, as no test can guarantee the complete absence of infectious agents.

✓ This kit contains components of animal origin. These materials should be handled as potentially infectious.

✓ Avoid contact with the acidic Stop Solution and Substrate Solution, which contains hydrogen peroxide and tetramethylbenzidine (TMB). Wear gloves and eye and clothing protection when handling these reagents. Stop and/or Substrate Solutions may cause skin/eyes irritation. In case of contact with the Stop Solution and the Substrate Solution wash skin/eyes thoroughly with water and seek medical attention, when necessary.

✓ The materials must not be pipetted by mouth.

- Technical hints

✓ Reagents with different lot numbers should not be mixed.

✓ Use thoroughly clean glassware.

✓ Use deionized (distilled) water, stored in clean containers.

✓ Avoid any contamination among samples and reagents. For this purpose, disposable tips should be used for each sample and reagent.

✓ Substrate Solution should remain colourless until added to the plate. Keep Substrate Solution protected from light.

✓ Stop Solution should remain colourless until added to the plate. The colour developed in the wells will turn from blue to yellow immediately after the addition of the Stop Solution. Wells that are green in colour indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.

✓ Dispose of consumable materials and unused contents in accordance with applicable national regulatory requirements

Assay Protocol

Reagent Preparation

- ✓ All reagents need to be brought to room temperature prior to use.
- ✓ Always prepare only the appropriate quantity of reagents for your test.
- ✓ Do not use components after the expiration date marked on their label.

- Assay reagents supplied ready to use:

✓ Antibody Coated Microtiter Strips

Stability and storage:

Return the unused strips to the provided aluminium zip-sealed bag with desicant and seal carefully. Remaining Microtiter Strips are stable 3 months stored at 2-8°C and protected from the moisture.

- ✓ Conjugate solution
- ✓ Dilution Buffer
- ✓ Substrate Solution
- ✓ Stop Solution

Stability and storage:

Opened reagents are stable 3 months when stored at 2-8°C.

- Assay reagents supplied concentrated or lyophilized:

✓ Human Leptin Receptor Master Standard

Refer to the Certificate of Analysis for current volume of Dilution Buffer needed for reconstitution!!!

Reconstitute the lyophilized Master Standard just prior to the assay. Let it dissolve at least 15 minutes with occasional gentle shaking (not to foam). The resulting concentration of human leptin receptor in the stock solution is 32 ng/mL.

Prepare set of standards using Dilution Buffer as follows:

Volume of Standard	Dilution Buffer	Concentration
Stock	-	32 ng/mL
250 µL of stock	250 µL	16 ng/mL
250 µL of 16 ng/mL	250 µL	8 ng/mL
250 µL of 8 ng/mL	250 µL	4 ng/mL
250 µL of 4 ng/mL	250 µL	2 ng/mL
250 µL of 2 ng/mL	250 µL	1 ng/mL

- ✓ Prepare Standard are ready to use, do not dilute them.

Stability and storage:

Do not store the stock solution, neither prepared Standard solutions.

- ✓ Quality Controls High, Low

  Refer to the Certificate of Analysis for current Quality Control concentration!!! Reconstitute each Quality Control (HIGH and LOW) with 350 µL of distilled (deionized) water just prior to the assay. Let it dissolve at least 15 minutes with occasional gentle shaking (not to foam). Dilute reconstituted Quality Controls 3x with Dilution Buffer, e.g. 50 µL of Quality Control + 100 µL of Dilution Buffer when assaying samples in singlets, or preferably 100 µL of Quality Control + 200 µL of Dilution Buffer for duplicates. Mix well (not to foam).

  Stability and storage:

  The reconstituted Quality Controls must be used immediately or aliquoted and frozen at -20°C for 3 month. Avoid repeated freeze/thaw cycles.

  Do not store the diluted Quality Controls.

  Note:

  Concentration of analyte in Quality Controls need not be anyhow associated with normal and/or pathological concentrations in serum or another body fluid. Quality Controls serve just for control that kit works in accordance with PDS and CoA and that ELISA test was carried out properly.

- ✓ Wash Solution Conc. (10x)

  Dilute Wash Solution Conc. (10x) ten fold in distilled water to prepare a 1x working solution. Example: 100 mL of Wash Solution Conc. (10x) + 900 mL of distilled water for use of all 96- wells.

  Stability and storage:

  The diluted Wash Solution is stable 1 month when stored at 2-8°C. Opened Wash Solution Conc. (10x) is stable 3 months when stored at 2-8°C.

Sample Preparation

The kit measures leptin receptor in serum and plasma (EDTA and heparin).

Samples should be assayed immediately after collection or should be stored at -20°C. Mix thoroughly thawed samples just prior to the assay and avoid repeated freeze/thaw cycles, which may cause erroneous results. Avoid using hemolyzed or lipemic samples.

Dilute samples 3x with the Dilution Buffer just prior to the assay, e.g. 50 µL of sample + 100 µL of Dilution Buffer for singlets, or preferably 100 µL of sample + 200 µL of Dilution Buffer for duplicates. Mix well (not to foam). Vortex is recommended.

Stability and storage:

Samples should be stored at -20°, or preferably at -70°C for long-term storage. Avoid repeated freeze/ thaw cycles.

Do not store the diluted samples.

Note: It is recommended to use a precision pipette and a careful technique to perform the dilution in order to get precise results.

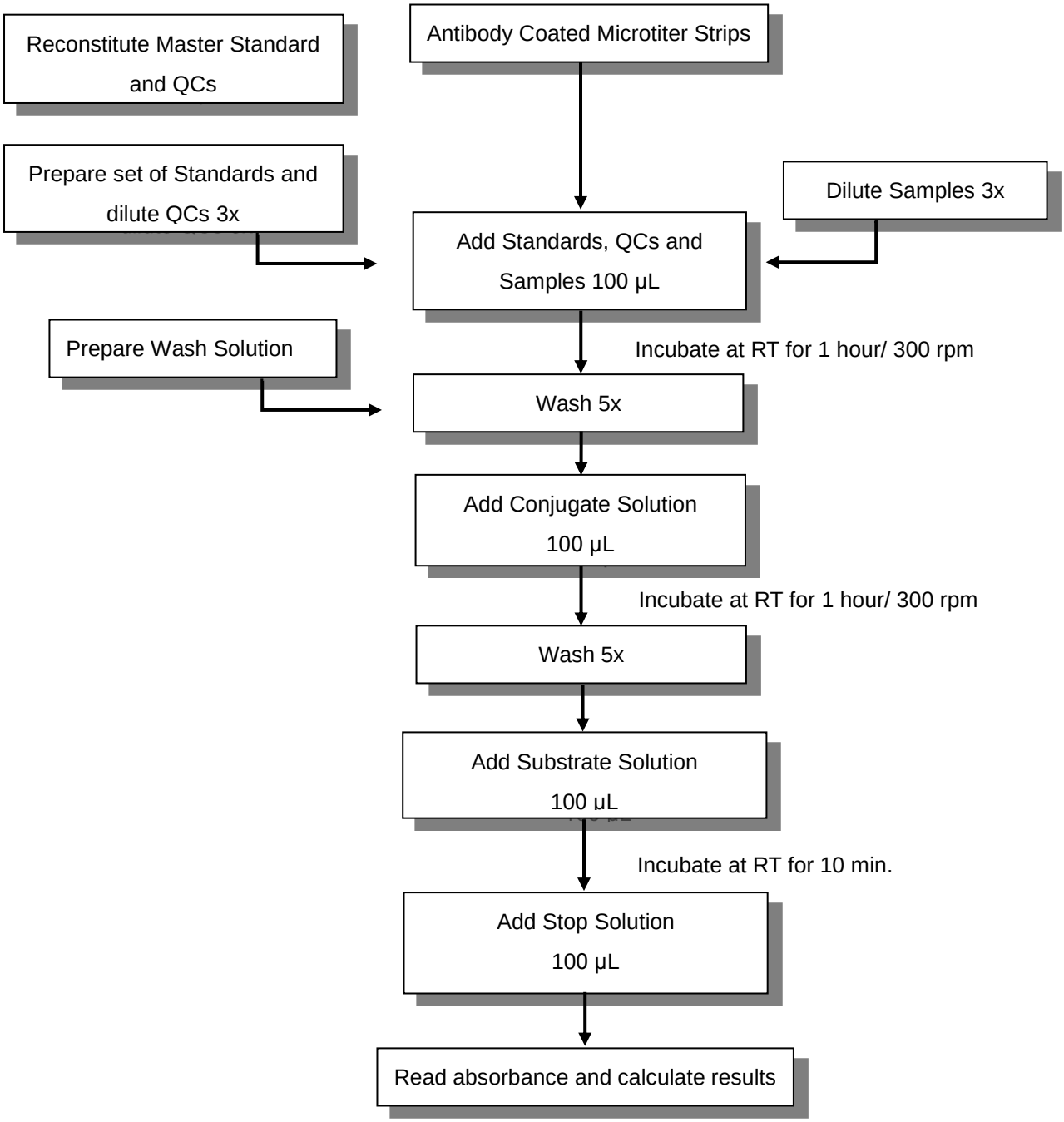
Assay Procedure

1. Pipet 100 µL of Standards, Quality Controls, Dilution Buffer (=Blank) and diluted samples, preferably in duplicates, into the appropriate wells. See Plate Layout for example of work sheet.
2. Incubate the plate at room temperature (ca. 25°C) for 1 hour, shaking at ca. 300 rpm on an orbital microplate shaker.
3. Wash the wells 5-times with Wash Solution (0.35 mL per well). After final wash, invert and tap the plate strongly against paper towel.
4. Add 100 µL of Conjugate Solution into each well.
5. Incubate the plate at room temperature (ca. 25°C) for 1 hour, shaking at ca. 300 rpm on an orbital microplate shaker.
6. Wash the wells 5-times with Wash Solution (0.35 mL per well). After final wash, invert and tap the plate strongly against paper towel.
7. Add 100 µL of Substrate Solution Conjugate into each well. Avoid exposing the microtiter plate to direct sunlight. Covering the plate with e.g. aluminium foil is recommended.
8. Incubate the plate for 10 minutes at room temperature. The incubation time may be extended [up to 20 minutes] if the reaction temperature is below than 20°C. Do not shake the plate during the incubation.
9. Stop the colour development by adding 100 µL of Stop Solution.
10. Determine the absorbance of each well using a microplate reader set to 450 nm, preferably with the reference wavelength set to 630 nm (acceptable range: 550 - 650 nm). Subtract readings at 630 nm (550 - 650 nm) from the readings at 450 nm. The absorbance should be read within 5 minutes following step 9.

Note: If some samples and standard/s have absorbances above the upper limit of your microplate reader, perform a second reading at 405 nm. A new standard curve, constructed using the values measured at 405 nm, is used to determine leptin receptor concentration of off-scale standards and samples. The readings at 405 nm should not replace the readings for samples that were "in range" at 450 nm.

Note 2: Manual washing: Aspirate wells and pipet 0.35 mL Wash Solution into each well. Aspirate wells and repeat twice. After final wash, invert and tap the plate strongly against paper towel. Make certain that Wash Solution has been removed entirely.

- Assay Procedure Summary



Data Analysis

Calculation of Results

Most microplate readers perform automatic calculations of analyte concentration. The standard curve is constructed by plotting the mean absorbance (Y) of Standards against the known concentration (X) of Standards in logarithmic scale, using the four-parameter algorithm. Results are reported as concentration of leptin receptor (ng/mL) in samples.

Alternatively, the logit log function can be used to linearize the standard curve, i.e. logit of the mean absorbance (Y) is plotted against log of the known concentration (X) of Standards.

The measured concentration of Quality Controls and samples calculated from the standard curve must be multiplied by their respective dilution factor because they have been diluted prior to the assay, e.g. 4 ng/mL (from standard curve) x 3 (dilution factor) = 12 ng/mL.

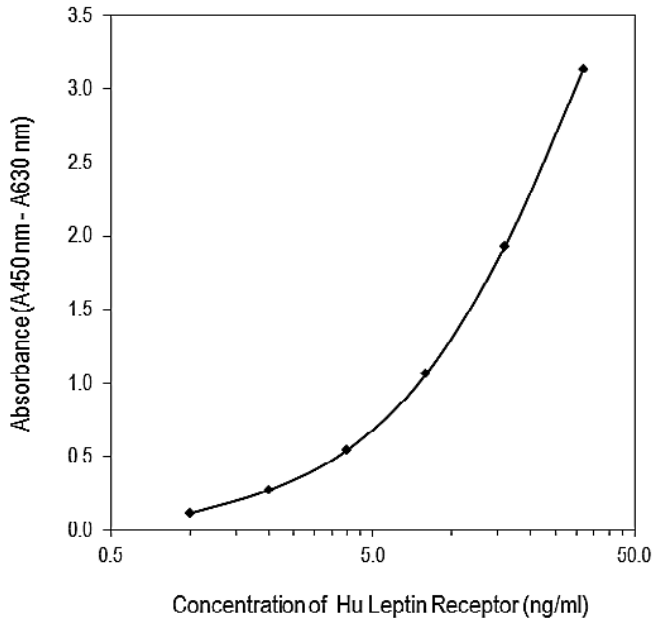


Figure 1: Typical Standard Curve for LEPR (Human) ELISA Kit

Performance Characteristics

- Sensitivity

  Limit of Detection (LOD) (defined as concentration of analyte giving absorbance higher than mean absorbance of blank* plus three standard deviations of the absorbance of blank: A_{blank} + 3xSD_{blank}) is calculated from the real leptin receptor values in wells and is 0.05 ng/mL.

  *Dilution Buffer is pipetted into blank wells.

- Limit of assay

  Samples with absorbances exceeding the absorbance of the highest standard should be measured again with higher dilution. The final concentration of sample calculated from the standard curve must be multiplied by the respective dilution factor.

- Specificity

  The antibodies used in this ELISA are specific for human leptin receptor with no detectable crossreactivities to human cytokines. Determination of leptin receptor does not interfere with haemoglobin (1.0 mg/mL), bilirubin (170 µmol/L) and triglycerides (5.0 mmol/L).

  Sera of several mammalian species were measured in the assay. See results below.

Mammalian serum sample	Observed crossreactivity
Bovine	no
Cat	no
Dog	no
Goat	no
Horse	no
Monkey	no
Mouse	no
Pig	no
Rabbit	no
Rat	no
Sheep	no

- Precision

  Intra-assay (Within-Run) (n=8)

Sample	Mean (ng/mL)	SD (ng/mL)	CV (%)
1	17.35	1.25	7.23
2	30.82	2.19	7.10

  Inter assay (Run-to-Run) (n=5)

Sample	Mean (ng/mL)	SD (ng/mL)	CV (%)
1	12.24	1.20	9.81
2	30.92	1.92	6.21

- Spiking Recovery

  Serum samples were spiked with different amounts of human leptin receptor and assayed.

Sample	Observed (ng/mL)	Expected (ng/mL)	Recovery O/E (%)
1	10.75	-	-
	15.30	15.92	96.1
	19.54	22.68	86.2
	21.14	25.97	81.4
2	16.52	-	-
	20.62	21.69	95.1
	25.23	28.45	88.7
	26.64	31.74	83.9

- Linearity

  Serum samples were serially diluted with Dilution Buffer and assayed.

Sample	Dilution	Observed (ng/mL)	Expected (ng/mL)	Recovery O/E (%)
1	-	34.57	-	-
	2x	19.49	17.29	112.8
	4x	10.03	8.64	116.1
	8x	4.30	4.32	99.5
2	-	28.15	-	-
	2x	14.78	14.08	105.0
	4x	8.01	7.04	113.8
	8x	3.52	3.52	100.0

- Effect of sample matrix

  EDTA, citrate and heparin plasmas were compared to respective serum samples from the same 10 individuals. However, we observed low correlation among serum and citrate plasma leptin receptor values. Results are shown below:

Volunteer No.	Serum (ng/mL)	Plasma (ng/mL)		
		EDTA	Citrate	Heparin
1	32.73	31.68	29.91	31.54
2	34.32	36.19	35.55	34.39
3	50.14	39.93	36.81	42.49
4	22.56	24.76	26.17	27.44
5	26.58	24.50	17.08	28.06
6	22.31	21.55	23.02	23.37
7	28.11	24.99	23.06	26.04
8	22.20	22.81	22.77	24.57
9	31.81	28.62	23.15	30.46
10	22.78	25.69	20.82	23.69
Mean (ng/mL)	29.35	28.07	25.83	29.21
Mean Plasma/Serum (%)	-	95.6	92.0	113.1
Coefficient of determination R^2	-	0.86	0.59	0.93

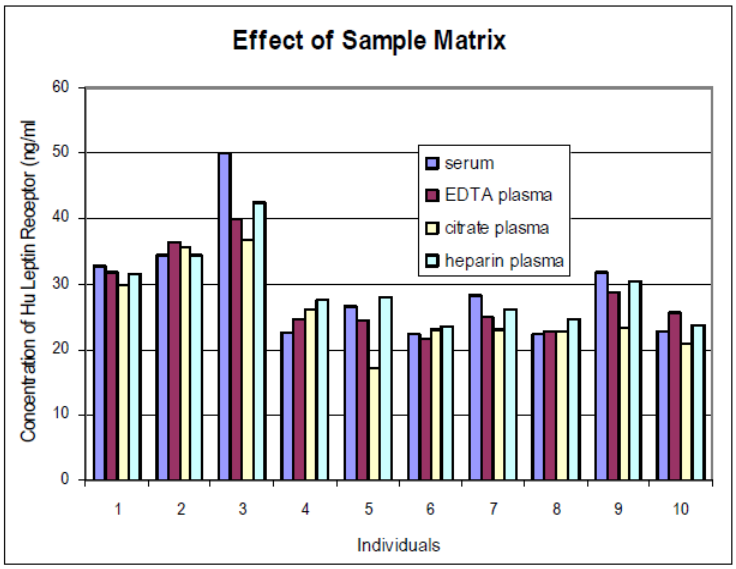


Figure 2: Leptin receptor levels measured using LEPR (Human) ELISA Kit from 10 individuals using serum, EDTA, citrate and heparin plasma, respectively.

- Stability of samples stored at 2-8°C

  Samples should be stored at -20°C. However, no decline in concentration of leptin receptor was observed in serum and plasma samples after 10 days when stored at 2-8°C. To avoid microbial contamination, samples were treated with e-aminocaproic acid and sodium azide, resulting in the final concentration of 0.03% and 0.1%, respectively.

Sample	Incubation Temp. Period	Serum (ng/mL)	Plasma (ng/mL)		
			EDTA	Citrate	Heparin
1	-20°C	49.75	41.48	38.82	35.69
	2-8°C, 1 day	47.13	43.03	41.38	41.19
	2-8°C, 10 days	45.04	44.28	46.02	40.49
2	-20°C	22.36	23.27	21.70	22.97
	2-8°C, 1 day	21.79	24.54	21.81	24.10
	2-8°C, 10 days	23.56	22.69	22.93	19.35
3	-20°C	33.28	33.34	35.09	34.46
	2-8°C, 1 day	35.80	33.49	30.82	32.29
	2-8°C, 10 days	35.15	33.96	31.77	35.45

- Effect of Freezing/Thawing

  No decline was observed in concentration of human leptin receptor in serum and plasma samples after repeated (5x) freeze/thaw cycles. However it is recommended to avoid unnecessary repeated freezing/thawing of the samples.

Sample	Number of f/t cycles	Serum (ng/mL)	Plasma (ng/mL)		
			EDTA	Citrate	Heparin
1	1x	21.39	20.89	16.77	21.79
	3x	18.69	18.31	16.20	22.72
	5x	20.06	20.47	16.43	21.15
2	1x	26.89	24.71	23.33	27.14
	3x	26.77	26.91	22.29	26.58
	5x	25.24	24.80	20.93	24.36
3	1x	18.07	18.51	16.00	18.96
	3x	17.57	17.95	17.19	19.73
	5x	19.39	18.55	17.29	19.62

- Reference range

  It is recommended that each laboratory include its own panel of control sample in the assay. Each laboratory should establish its own normal and abnormal reference ranges for leptin receptor levels with the assay.

- Definition of the standard

  The standard used in this kit is recombinant fusion protein chimera, which is composed of human IgG-Fc-fragment and human OB-R and is different from the native soluble OB-R that is measured in human serum. As a result of glycosylation, recombinant human OB-R/Fc chimera migrates as a 155-175 kDa protein in SDS-PAGE.

- Method comparison

  The LEPR (Human) ELISA has not been compared to the other commercial immunoassays.

Resources

Troubleshooting

- Weak signal in all wells

Possible explanations:

✓ Omission of a reagent or a step
✓ Improper preparation or storage of a reagent
✓ Assay performed before reagents were allowed to come to room temperature
✓ Improper wavelength when reading absorbance

- High signal and background in all wells

Possible explanations:

✓ Improper or inadequate washing
✓ Overdeveloping; incubation time with Substrate Solution should be decreased before addition of Stop Solution
✓ Incubation temperature over 30°C

- High coefficient of variation (CV)

Possible explanation:

✓ Improper or inadequate washing
✓ Improper mixing Standards, Quality Controls or samples

References

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Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	Standard 32	Standard 32	Blank	Blank	Sample 8	Sample 8	Sample 16	Sample 16	Sample 24	Sample 24	Sample 32	Sample 32
B	Standard 16	Standard 16	Sample 1	Sample 1	Sample 9	Sample 9	Sample 17	Sample 17	Sample 25	Sample 25	Sample 33	Sample 33
C	Standard 8	Standard 8	Sample 2	Sample 2	Sample 10	Sample 10	Sample 18	Sample 18	Sample 26	Sample 26	Sample 34	Sample 34
D	Standard 4	Standard 4	Sample 3	Sample 3	Sample 11	Sample 11	Sample 19	Sample 19	Sample 27	Sample 27	Sample 35	Sample 35
E	Standard 2	Standard 2	Sample 4	Sample 4	Sample 12	Sample 12	Sample 20	Sample 20	Sample 28	Sample 28	Sample 36	Sample 36
F	Standard 1	Standard 1	Sample 5	Sample 5	Sample 13	Sample 13	Sample 21	Sample 21	Sample 29	Sample 29	Sample 37	Sample 37
G	QC HIGH	QC HIGH	Sample 6	Sample 6	Sample 14	Sample 14	Sample 22	Sample 22	Sample 30	Sample 30	Sample 38	Sample 38
H	QC LOW	QC LOW	Sample 7	Sample 7	Sample 15	Sample 15	Sample 23	Sample 23	Sample 31	Sample 31	Sample 39	Sample 39