HUMAN LEPTIN RECEPTOR

ENZYME IMMUNOASSAY KIT

catalogue # A05175

96 wells

TABLE OF CONTENTS

For research laboratory use only.
Not for diagnostic use.

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HUMAN LEPTIN RECEPTOR EIA KIT

96 wells
Storage: 2-8℃
Expiry date: stated on the package

This kit contains:

☞A covered 96 well plate, pre-coated with a monoclonal anti-Leptin receptor antibody, ready to use
☞One vial of anti-Leptin receptor tracer, ready to use
☞Six vials of Leptin receptor standards, (2, 5, 10, 20, 50, 100 ng/mL), ready to use
☞Two vials of Quality Controls: low and high, ready to use
☞One vial of EIA buffer, ready to use
☞One vial of Substrate solution (TMB), ready to use
☞One vial of Stop solution (0.2 M H_2SO_4), ready to use
☞One vial of concentrated Wash buffer (10x), liquid
☞One instruction booklet
☞One template sheet
☞Three well cover sheet

Each kit contains sufficient reagents for 96 wells. This allows for the construction of one standard curve in duplicate and the assay of 41 samples in duplicate.

PRECAUTIONS FOR USE

Users are recommended to read all instructions for use before starting work.

Each time a new pipet tip is used, aspirate a sample of reagent and dispense into the same vessel. Repeat this operation two or three times before distribution.

For research laboratory use only.
Not for diagnostic use.
Do not pipet liquids by mouth.
Do not use kit components beyond the expiration date.
Do not mix different lot numbers.
Do not eat, drink, or smoke in area in which kit reagents are handled.
Avoid splashing.

This kit contains components of human origin. These materials were found non-reactive for HbsAg, HCV antibody and for HIV 1/2 antibody and antigen. However, these materials should be handled as potentially infectious, as no test can guarantee the complete absence of infectious agents. Wear gloves and laboratory coats are recommended when handling immunodiagnostics materials and samples of human origin.

Stop solution and Substrate solution are potential harmful solution. To avoid any contact, wear eye, hand, face and clothing protection when handling these reagents.

PRINCIPLE OF THE ASSAY

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 comprising 816 amino acid residues. 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.

This Enzyme Immunometric Assay (EIA) is based on a double-antibody sandwich technique. The wells of the plate supplied with the kit are coated with a monoclonal antibody specific of human Leptin receptor. This antibody will bind any Leptin receptor introduced in the wells (sample or standard).

An horseradish peroxydase (HRP) conjugated monoclonal antibody which binds selectively to different epitopes on the leptin receptor molecule, is also added to the wells.

This allows the two antibodies to form a sandwich by binding on different parts of the human leptin receptor molecule.

The sandwich is immobilised on the plate so the excess reagents may be washed away. The concentration of the human leptin receptor is then determined by measuring the enzymatic activity of the HRP using the hydrogen peroxide/TMB solution. The reaction is stopped by addition of sulfuric acid solution. The HRP tracer acts on TMB Reagent to form a yellow compound.

The intensity of the colour, which is determined by spectrophotometry, is proportional to the amount of the human Leptin receptor present in the well during the immunological incubation.

The principle of the assay is summarised below:
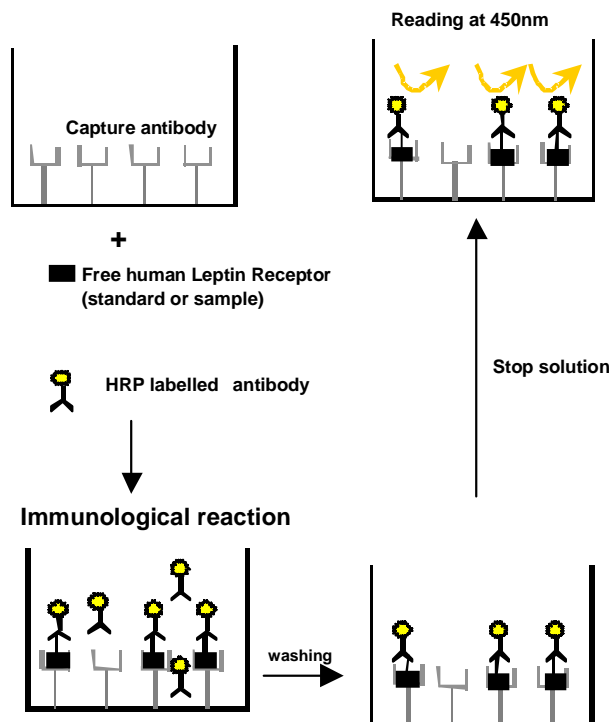
MATERIALS AND EQUIPMENT REQUIRED

In addition to standard laboratory equipment, the following material is required:

FOR THE ASSAY

☞Precision micropipettes (10 to 1000 µL)

☞Spectrophotometer plate reader (450 nm +/- 10 nm filter)

☞Microtitration washer (or washbottles)

☞Microplate shaker

☞Multichannel pipette 50-200 µL and disposable tips

☞Distilled or deionised water

☞Polypropylene tubes

SAMPLE PREPARATION

This assay may be used to measure human Leptin receptor in human samples such as serum, plasma and culture supernatant

GENERAL PRECAUTIONS

☞All samples must be free of organic solvents prior to assay.

☞Samples should be assayed immediately after collection or should be stored at -20℃.

SAMPLE PREPARATION

☞No prior extraction procedure is necessary.

☞To measure human Leptin receptor, dilute serum or plasma samples 1/3 in EIA buffer (i.e. 100 µL sample + 200 µL EIA buffer).
Do not store the diluted samples.

REAGENT PREPARATION

All reagents need to be brought to room temperature prior to the assay. Assay reagents are supplied ready to use, except the concentrated Wash buffer.

☞Human Leptin receptor standard
Dilute standards 1/3 in EIA buffer prior to use (i.e. 100 µL standard + 200 µL EIA buffer).
The undiluted standards could be frozen at -20℃ un til next use.
Do not store the diluted (1/3) standards.

No decline was observed in concentration of human Leptin receptor in serum and plasma samples after repeated (5x) freezing/thawing cycles. However, repeated thawing-freezing cycles should be avoided.

☞Quality Controls
Reconstitute each vial of Quality Control with 350 µL of distilled or deionised water at least 15 minutes prior the assay.
Dilute Quality Control 1/3 in EIA buffer prior to use (i.e. 100 µL QC + 200 µL EIA buffer).
Quality Control is stable until the expiration date (see label on the box) when stored at -20℃.

☞Wash buffer
Dilute 100 mL of concentrated Wash buffer (100 mL, 10x) to 1000 mL with distilled or deionised water.
The diluted Wash buffer is stable for one month when stored at 2-8℃.

☞Hydrogen peroxide/TMB solution
Substrate solution should remain colourless until added to the plate. Keep substrate solution protected from the light.

ASSAY PROCEDURE

It is recommended to perform the assays in duplicate and to follow the instructions hereafter.

DISTRIBUTION OF REAGENTS AND SAMPLES

A plate set-up is suggested below. The content of each well may be recorded on the sheet provided with the kit.
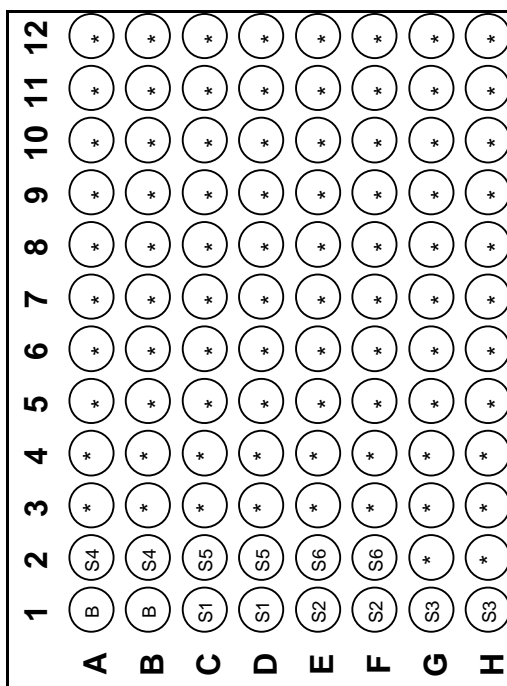
PIPETTING THE REAGENTS

All samples and reagents must reach room temperature prior performing the assay. Use different pipet tips to pipet the buffer, standard, sample, tracer and other reagents.

DISTRIBUTION OF REAGENTS AND SAMPLES

✎ Human Leptin receptor standard:
  Dispense 100 µL of the six diluted standards (S1 to S6) in duplicate to appropriated wells. Start with the lowest concentration standard and equilibrate the tip in the next higher standard before pipetting.

✎ Quality Control and samples:
  Dispense in duplicate 100 µL of diluted Quality Controls, and samples to appropriate wells.

✎ EIA buffer:
  Dispense in duplicate 100 µL to blank (B) wells.

	1	2	3	4	5	6	7	8	9	10	11	12
A	B	S4	*	*	*	*	*	*	*	*	*	*
B	B	S4	*	*	*	*	*	*	*	*	*	*
C	S1	S5	*	*	*	*	*	*	*	*	*	*
D	S1	S5	*	*	*	*	*	*	*	*	*	*
E	S2	S6	*	*	*	*	*	*	*	*	*	*
F	S2	S6	*	*	*	*	*	*	*	*	*	*
G	S3	*	*	*	*	*	*	*	*	*	*	*
H	S3	*	*	*	*	*	*	*	*	*	*	*

B: Blank
S1-S6: Standards 1-6
*: Samples or Quality Controls

INCUBATING THE PLATE

✎ Cover the plate with the cover sheet and incubate at room temperature for 1 hour, shaking at 300 rpm on an orbital microplate shaker.

✎ Rinse each well 5 times with Wash buffer (350 µL/well). Slightly shake the plate for 5 minutes (with orbital shaker). Dry by inversion on absorbent paper.

✎ Anti-Leptin receptor tracer:
  Dispense 100 µL to each well.

✎ Cover the plate with the cover sheet and incubate at room temperature for 1 hour, shaking at 300 rpm on an orbital microplate shaker.

✎ Rinse each well 5 times with Wash buffer (350 µL/well). Slightly shake the plate for 5 minutes (with orbital shaker). Dry by inversion on absorbent paper.

DEVELOPING AND READING THE PLATE

✎ Dispense 100 µL of Substrate solution to the 96 wells. Incubate the plate in the dark during 10 minutes at room temperature. Avoid exposure to direct sunlight. It is recommended to cover the plate with aluminium foil.

✎ Stop the colour development by adding 100 µL of Stop Solution.

✎ Read the absorbance at 450 nm within 5 minutes following Stop solution addition.

*Note: If the microplate reader is not capable of reading absorbance greater than the absorbance of the lowest standard (the highest absorbance of the calibration curve), 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 samples. **The readings at 405 nm should not replace the on-scale readings at 450 nm.***

Enzyme Immunoassay Protocol (Volumes are in µL)	Blank	Standard	Sample
EIA Buffer	100	-	-
Standard	-	100	-
Sample	-	-	100
Incubate the plate at room temperature for 1 hour			
Wash the plate 5 times			
Tracer	-	100	100
Incubate the plate at room temperature for 1 hour			
Wash the plate 5 times			
TMB solution	100	100	100
Incubate the plate in the dark at room temperature during 10 minutes			
Stop solution	100	100	100
Read the plate at 450 nm			

DATA ANALYSIS

Make sure that your plate reader has substracted the absorbance readings of the blank well (absorbance of TMB solution) from the absorbance readings of the rest of the plate. If not, do it now.

- ✎ Using a semi-log graph paper, plot the absorbance for each standard (y axis) versus concentration (x axis) of standards. Draw a best-fit line through the points.

- ✎ To determine the concentration of your samples, find the absorbance value on the y axis. Read the corresponding value on the x axis which is the concentration of your unknown sample. Samples with a concentration greater than 100 ng/mL should be re-assayed after dilution.

- ✎ Most plate readers are supplied with curve-fitting software capable of graphing this type of data (logit/log or 4-parameter). If you have this type of software, we recommend using it. Refer to it for further information.

TYPICAL DATA

EXAMPLE DATA

The following data are for demonstration purposes only. Your data may be different but still correct. These data were obtained using all reagents supplied in this kit according to the protocol. A 4-parameter curve fitting was used to determine the concentrations.

Human Leptin receptor	mAU
Blank	52
Standard 100 ng/mL	3 343
Standard 50 ng/mL	2 176
Standard 20 ng/mL	1 047
Standard 10 ng/mL	590
Standard 5 ng/mL	326
Standard 2 ng/mL	156
QC High	1 693
QC Low	969

ACCEPTABLE RANGE

☞QC samples: see label on the vials.

HUMAN LEPTIN RECEPTOR STANDARD CURVE
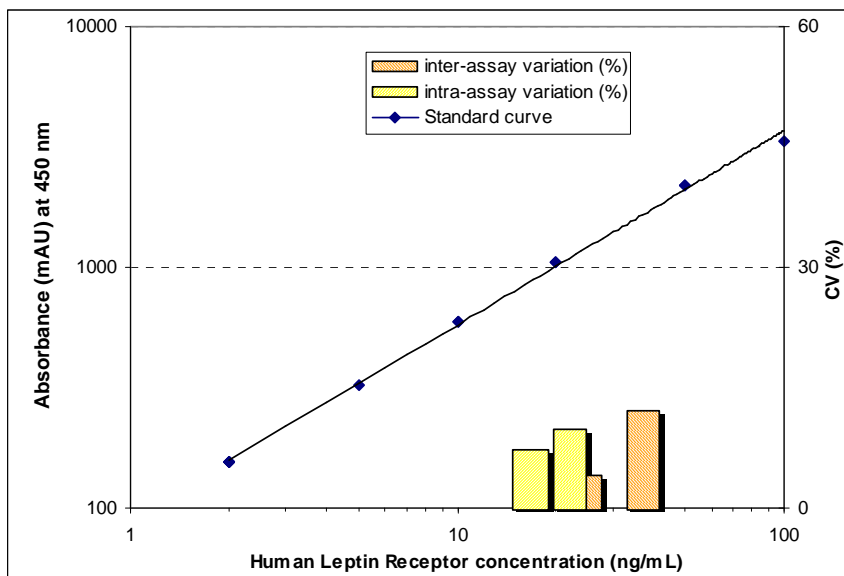
ASSAY VALIDATION AND CHARACTERISTICS

This assay may be used to measure human Leptin receptor in human samples such as serum, plasma and culture supernatant.

☞Cross-reactivity:

Mouse Leptin	<0.1%
Rat Leptin	<0.1%
Bovine Leptin	<0.1%
Rabbit Leptin	<0.1%
Horse Leptin	<0.1%
Goat Leptin	<0.1%
Sheep Leptin	<0.1%
Pig Leptin	<0.1%

☞Sensitivity:

The limit of detection (defined as such a concentration of human Leptin Receptor giving absorbance higher than mean absorbance of blank* plus three standard deviations of the absorbance of blank: A_{blank} + $3*SD_{blank}$) is better than 0.4 ng/mL. The EIA buffer was pipetted into blank wells, and the microtiter plate is blanked on air.

☞Precision:
- Intra-assay variation (n=8)

Sample	Mean (ng/mL)	Standard Deviation (ng/mL)	CV (%)
1	20.04	2.09	10.1
2	23.19	1.70	7.4
3	18.12	1.17	6.5
4	21.26	2.05	9.7

- Inter-assay variation (n=7)

Sample	Mean (ng/mL)	Standard Deviation (ng/mL)	CV (%)
1	38.51	3.80	9.9
2	17.43	1.26	7.2
3	21.25	0.89	4.2
4	25.19	1.39	5.5

☞Recovery test:
Serum samples were spiked with different amounts of human Leptin receptor and assayed.

Sample	Observed (ng/mL)	Expected (ng/mL)	Recovery O/E (%)
1	18.25	-	-
	26.66	29.19	91.3
	32.79	37.77	86.8
	42.36	46.64	90.8
2	21.35	-	-
	28.33	32.29	87.8
	36.60	40.87	89.6
	42.80	49.74	86.0
3	13.26	-	-
	23.47	23.90	98.2
	33.33	32.48	102.6
	42.34	40.94	103.4
4	20.74	-	-
	28.57	31.38	91.0
	38.64	39.96	92.2
	45.19	48.42	93.3

☞Dilution test:

Sample	Dilution	Observed (ng/mL)	Expected (ng/mL)	Recovery O/E (%)
1	-	45.43	-	-
	1:2	20.64	22.72	90.9
	1:4	9.91	11.36	87.3
	1:8	4.48	5.68	78.9
2	-	26.47	-	-
	1:2	13.74	13.24	103.8
	1:4	7.74	6.62	116.9
	1:8	3.51	3.31	106.0

☞Serum/Plasma sample:

Samples from 10 healthy individuals were taken and treated by different methods, results shown below:

Sample (n=16)	Mean (ng/mL)	Plasma / Serum (%)
Serum	29.35	-
Plasma - Heparin	29.21	113.1
Plasma - Citrate	25.83	92.0
Plasma - EDTA	28.07.	95.6

☞Stability of samples at 4℃:

Samples should be stored at -20℃. However, no decl ine was observed in concentration of human Leptin receptor in serum and plasma samples when stored at 4℃ for 10 days. To avoid microbial contamination, add NaN3 to a final concentration 0.1% to the samples.

Sample	Temp /Time	Serum (ng/mL)	Plasma (ng/mL)		
			Heparin	Citrate	EDTA
1	-	33.28	33.34	34.46	35.09
	4℃/1 day	35.80	33.49	32.30	30.82
	4℃/10 days	35.15	33.96	35.45	31.77
2	-	49.75	41.48	35.69	38.82
	4℃/1 day	47.13	43.00	41.19	41.38
	4℃/10 days	45.04	44.28	40.49	46.02
3	-	22.36	23.27	22.97	21.70
	4℃/1 day	21.80	21.54	24.11	21.81
	4℃/10 days	23.56	22.70	19.34	22.93

ASSAY TROUBLE SHOOTING

☞Absorbance values too low:
- One reagent has not been dispensed
- Incorrect preparation or reagent storage
- Assay performed before reagents reach room temperature

☞High signal and background in all wells:
- Inefficient washing
- Overdeveloping; incubation time should be reduced before adding Stop Solution

☞High dispersion of duplicates:
- Poor pipetting technique or irregular plate washing.

These are a few examples of problems that may occur. If you need further assistance, SPI-BIO will be happy to answer any questions or information about this assay. Please feel free to contact our technical support staff by letter, phone (33 (0)1 39 30 62 60), fax (33 (0)1 39 30 62 99) or E-mail (sales@spibio.com), and be sure to indicate the lot number of the kit (see outside of the box).

SPI-BIO offers a training workshop in EIA practice & theory. This workshop is given twice a year. For further information, please contact our Customer Relation Representative (33 (0)1 39 30 62 60).

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