Quantikine® ELISA

Human Leptin R Immunoassay

Catalog Number DOBR00

For the quantitative determination of human Leptin Receptor (Leptin R) concentrations in cell culture supernates, serum, and plasma.

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

Leptin is a hormone released primarily by adipose tissues and is known for its putative roles in the regulation of food intake and energy metabolism (1-3). Leptin is multi-functional, as well, with involvement in other physiological processes including angiogenesis, reproduction, hematopoiesis, bone metabolism, and immune function (1-8). Its activities are mediated via the Leptin receptor (Leptin R or OB R). Leptin R is a member of the type I cytokine receptor family and has significant amino acid (aa) sequence identity with gp130, G-CSF R, and LIF R (9). Human Leptin R exhibits approximately 78% aa sequence identity with its murine counterpart (10). The gene encodes a precursor protein with a signal peptide, a multi-domain extracellular region (cytokine receptor homology (CRH) domains and fibronectin type III domains (WSXWS motif)), a transmembrane domain, and a cytoplasmic domain containing box motifs capable of supporting JAK/STAT signaling (11, 12). Multiple isoforms of human and rodent Leptin R have been described including a long form (OB RL or OB Rb), at least four shorter isoforms with truncated cytoplasmic domains (OB Ra, Rc, Rd, and Rf), and a soluble variant that is thus far described only in rodents (OB Re) (13). Soluble forms found in humans may be generated post-translationally via metalloprotease-mediated ectodomain shedding (14).

There are distinct differences in the expression patterns and activities of the Leptin R variants. OB RL is highly expressed in the hypothalamus and is thought to be the mediator of Leptin effects on satiety (10, 15). In addition to JAK/STAT, other signaling mediators of the receptor include IRS, PI3-kinase, MAP kinase, and AMP kinase (8, 16). Leptin stimulates the expression of suppressor of cytokine signaling 3 (SOCS-3), which may act as a negative feedback regulator of Leptin R function (17, 18). The shorter isoforms with truncated cytoplasmic domains exhibit limited signaling capability and their functions are less clear (11), however, they have been implicated in Leptin transport across the blood-brain barrier (19-21).

Leptin R is the primary Leptin-binding protein in human blood (22). Although functions of the receptor continue to be elucidated, it may act as a negative regulator of Leptin activity, or it may maintain a pool of available bioactive Leptin by binding and delaying its clearance from circulation (23-25). In humans, Leptin R levels are inversely proportional to adiposity and are elevated in females versus males (26-30). In addition, levels are highest in infants, decrease into adolescence, and remain relatively stable throughout adulthood (29, 31). Leptin R is also found upregulated in patients with chronic heart failure, end-stage renal disease, and anorexia (32-35).

The Quantikine® Human Leptin R Immunoassay is a 4.5 hour solid-phase ELISA designed to measure human Leptin R in cell culture supernates, serum, and plasma. It contains NS0-expressed recombinant human Leptin R/Fc Chimera and has been shown to accurately quantitate the recombinant factor. Results obtained using natural human Leptin R showed linear curves that were parallel to the standard curves obtained using the Quantikine® kit standards. These results indicate that this kit can be used to determine relative mass values for natural human Leptin R.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human Leptin R has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any Leptin R present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked monoclonal antibody specific for human Leptin R is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of Leptin R bound in the initial step. The color development is stopped and the intensity of the color is measured.

LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- If samples generate values higher than the highest standard, further dilute the samples with the appropriate calibrator diluent and repeat the assay.
- Any variation in diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- Variations in sample collection, processing, and storage may cause sample value differences.
- This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the Quantikine® Immunoassay, the possibility of interference cannot be excluded.

TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

PART	PART #	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Human Leptin R Microplate	892685	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human Leptin R.	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.*
Human Leptin R Conjugate	892686	21 mL of a monoclonal antibody specific for human Leptin R conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Human Leptin R Standard	892687	Recombinant human Leptin R/Fc Chimera in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	
Assay Diluent RD1W	895117	11 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD5P	895151	21 mL of a concentrated buffered protein base with preservatives. *For cell culture supernate samples.*	
Calibrator Diluent RD6Q	895128	21 mL of animal serum with preservatives. *For serum/plasma samples.*	
Wash Buffer Concentrate	895003	21 mL of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Color Reagent A	895000	12 mL of stabilized hydrogen peroxide.	
Color Reagent B	895001	12 mL of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895032	6 mL of 2 N sulfuric acid.	
Plate Sealers	N/A	4 adhesive strips.	

* Provided this is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 500 mL graduated cylinder.
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm.
- Test tubes for dilution of standards and samples.
- Human Leptin R Controls (optional; R&D Systems®, Catalog # QC112).

PRECAUTIONS

Calibrator Diluent RD6Q contains sodium azide which may react with lead and copper plumbing to form explosive metallic azides. Flush with large volumes of water during disposal.

The Stop Solution provided with this kit is an acid solution.

Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.

Color Reagent B may cause skin, eye, and respiratory irritation. Avoid breathing fumes.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Refer to the MSDS on our website prior to use.

SAMPLE COLLECTION & STORAGE

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: *Citrate plasma has not been validated for use in this assay.*

SAMPLE PREPARATION

Serum and plasma samples require a 5-fold dilution. A suggested 5-fold dilution is 50 µL of sample + 200 µL of Calibrator Diluent RD6Q.

REAGENT PREPARATION

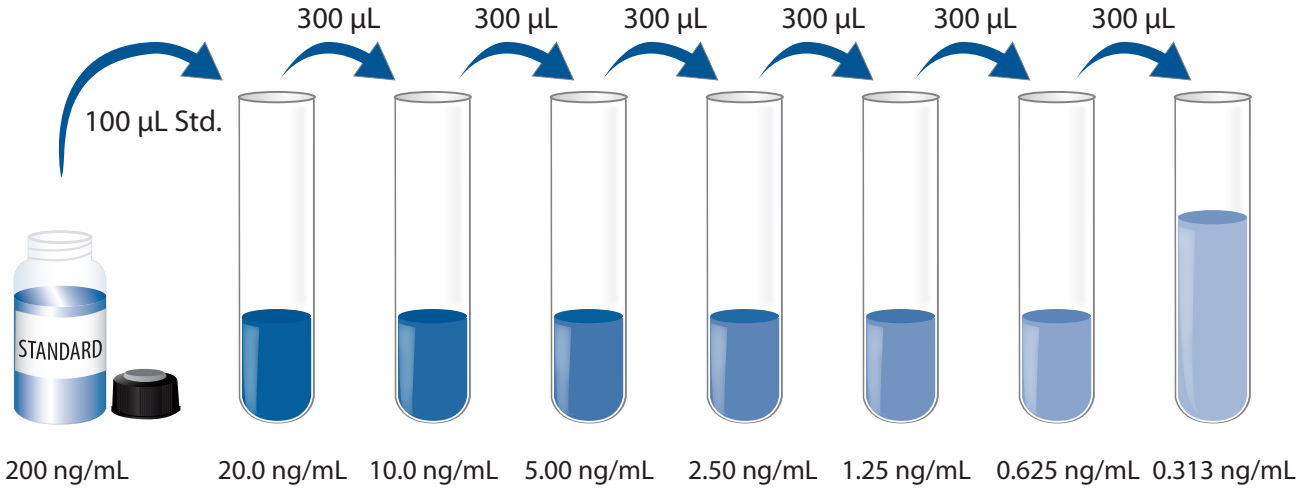
Bring all reagents to room temperature before use.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 500 mL of Wash Buffer.

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 200 µL of the resultant mixture is required per well.

Human Leptin R Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human Leptin R Standard with deionized or distilled water. This reconstitution produces a stock solution of 200 ng/mL. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 900 µL of Calibrator Diluent RD5P (*for cell culture supernate samples*) or Calibrator Diluent RD6Q (*for serum/plasma samples*) into the 20.0 ng/mL tube. Pipette 300 µL of the appropriate calibrator diluent into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 20.0 ng/mL standard serves as the high standard. The appropriate calibrator diluent serves as the zero standard (0 ng/mL).



ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all samples, controls, and standards be assayed in duplicate.

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 100 µL of Assay Diluent RD1W to each well.
4. Add 50 µL of standard, control, or sample* per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature on a horizontal orbital microplate shaker (0.12" orbit) set at 500 ± 50 rpm.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (400 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 200 µL of Human Leptin R Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature on the shaker.
7. Repeat the aspiration/wash as in step 5.
8. Add 200 µL of Substrate Solution to each well. **Protect from light.**
   For Cell Culture Supernate Samples: Incubate for **20 minutes** at room temperature **on the benchtop.**
   For Serum/Plasma Samples: Incubate for **30 minutes** at room temperature **on the benchtop.**
9. Add 50 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

*Samples may require dilution. See Sample Preparation section.

CALCULATION OF RESULTS

Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

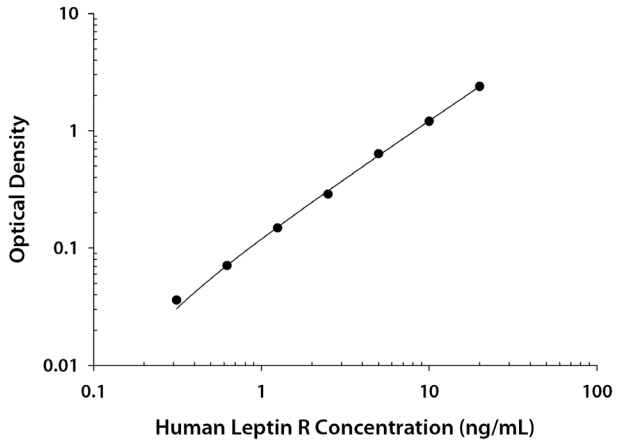
Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the human Leptin R concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL DATA

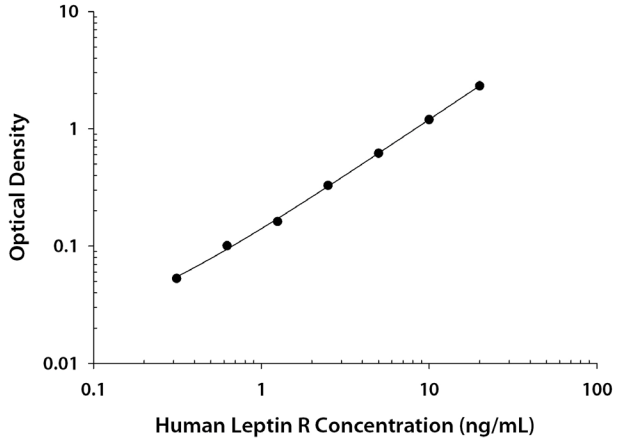
These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

CELL CULTURE SUPERNATE ASSAY



(ng/mL)	O.D.	Average	Corrected
0	0.042 0.044	0.043	—
0.313	0.077 0.081	0.079	0.036
0.625	0.112 0.115	0.114	0.071
1.25	0.192 0.192	0.192	0.149
2.50	0.323 0.341	0.332	0.289
5.00	0.658 0.684	0.671	0.638
10.0	1.250 1.250	1.250	1.207
20.0	2.410 2.460	2.435	2.392

SERUM/PLASMA ASSAY



(ng/mL)	O.D.	Average	Corrected
0	0.053 0.059	0.056	—
0.313	0.100 0.117	0.109	0.053
0.625	0.142 0.172	0.157	0.101
1.25	0.211 0.224	0.218	0.162
2.50	0.384 0.386	0.385	0.329
5.00	0.665 0.685	0.675	0.619
10.0	1.250 1.260	1.255	1.199
20.0	2.350 2.440	2.395	2.339

PRECISION

Intra-assay Precision (Precision within an assay)
Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays)
Three samples of known concentration were tested in forty separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of components.

CELL CULTURE SUPERNATE ASSAY

	Intra-Assay Precision			Inter-Assay Precision		
Sample	**1**	**2**	**3**	**1**	**2**	**3**
n	20	20	20	40	40	40
Mean (ng/mL)	1.83	5.15	11.2	1.69	5.09	10.7
Standard deviation	0.071	0.283	0.292	0.097	0.282	0.633
CV (%)	3.9	5.5	2.6	5.7	5.5	5.9

SERUM/PLASMA ASSAY

	Intra-Assay Precision			Inter-Assay Precision		
Sample	**1**	**2**	**3**	**1**	**2**	**3**
n	20	20	20	40	40	40
Mean (ng/mL)	2.34	7.01	14.6	2.34	6.95	14.3
Standard deviation	0.142	0.155	0.709	0.202	0.370	0.966
CV (%)	6.1	2.2	4.9	8.6	5.3	6.8

RECOVERY

The recovery of human Leptin R spiked to levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	96	91-102%
Serum* (n=4)	106	95-114%
EDTA plasma* (n=4)	104	92-115%
Heparin plasma* (n=4)	103	92-113%

*Samples were diluted prior to assay as directed in the Sample Preparation section.

SENSITIVITY

Eighty assays were evaluated and the minimum detectable dose (MDD) of human Leptin R ranged from 0.020-0.128 ng/mL. The mean MDD was 0.057 ng/mL.

The MDD was determined by adding two standard deviations to the mean O.D. value of twenty zero standard replicates and calculating the corresponding concentration.

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of human Leptin R were diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

		Cell culture media (n=4)	Serum* (n=4)	EDTA plasma* (n=4)	Heparin plasma* (n=4)
1:2	Average % of Expected	103	103	104	105
	Range (%)	102-105	99-108	101-108	102-108
1:4	Average % of Expected	101	108	109	104
	Range (%)	101-102	102-114	105-111	102-107
1:8	Average % of Expected	103	106	109	105
	Range (%)	98-109	93-112	105-114	96-110
1:16	Average % of Expected	92	105	105	105
	Range (%)	88-95	93-111	101-114	84-115

*Samples were diluted prior to assay as directed in the Sample Preparation section.

CALIBRATION

This immunoassay is calibrated against a highly purified NS0-expressed recombinant human Leptin R/Fc Chimera produced at R&D Systems®.

SAMPLE VALUES

Serum/Plasma - Samples from apparently healthy volunteers were evaluated for the presence of human Leptin R in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean (ng/mL)	Range (ng/mL)	Standard Deviation (ng/mL)
Serum (n=37)	28.2	13.1-55.4	7.59
EDTA plasma (n=37)	25.0	12.6-49.6	6.96
Heparin plasma (n=37)	24.9	12.4-49.3	7.33

Cell Culture Supernates - Human peripheral blood cells (1 x 10^6 cells/mL) were cultured in DMEM supplemented with 5% fetal bovine serum, 50 µM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. Cells were cultured unstimulated or stimulated with 10 µg/mL PHA. Aliquots of the cell culture supernate were removed and assayed for levels of human Leptin R. No detectable levels were observed.

SPECIFICITY

This assay recognizes natural and recombinant human Leptin R.

The factors listed below were prepared at 200 ng/mL in calibrator diluent and assayed for cross-reactivity. Preparations of the following factors at 200 ng/mL in a mid-range recombinant human Leptin R control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:	Recombinant mouse:
Leptin	Leptin
LIF	Leptin R
RELM β	LIF
Resistin	RELM α
	Resistin

Recombinant rat Leptin interferes at concentrations > 10.0 ng/mL.

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