Quantikine® ELISA

Mouse/Rat Leptin Immunoassay

Catalog Number MOB00B
SMOB00B
PMOB00B

For the quantitative determination of mouse or rat Leptin concentrations in cell culture supernates, tissue homogenates, 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

Mouse Leptin is a protein product of the mouse *obese* gene (1, 2). Mutant mice that lack functional Leptin have been found to be obese, diabetic, infertile and to have reduced activity, metabolism and body temperature. cDNA clones encoding Leptin have been isolated from human, simian, mouse and rat cells. Mature mouse Leptin shares approximately 96% and 84% amino acid (aa) sequence identity with the rat and human proteins, respectively. Mouse Leptin cDNA encodes a 167 aa residue protein with a 21 aa residue signal sequence that is cleaved to yield the 146 aa residue mature protein. The expression of Leptin mRNA has been shown to be restricted to adipose tissues and placenta (2).

A high-affinity receptor for Leptin (OB-R) with homology to gp130, G-CSF receptor, and LIF receptor has been cloned (3). Multiple isoforms of OB-R, including a long form (OB-R_L) with a large cytoplasmic domain capable of signal transduction, and several receptor isoforms with short cytoplasmic domains (OB-R_S) lacking signal transducing capabilities, have been identified (4-6). An OB-R transcript lacking a transmembrane domain and potentially encoding a soluble form of the receptor has also been described (7). OB-R_L transcripts were reported to be expressed predominantly in regions of the hypothalamus previously thought to be important in body weight regulation. Expression of OB-R_S transcripts have been found in multiple tissues, including the choroid plexus, lung, kidney and primitive hematopoietic cell populations (2). OB-R has been shown to be encoded by the mouse diabetes (*db*) and rat fatty (*fa*) genes (8). Rodents homozygous for the *db* or *fa* mutations have long been known to exhibit an obesity phenotype almost identical to the phenotype of *ob/ob* mice (9).

High Leptin levels (ng/mL) have been detected in mouse, rat and human serum or plasma. Circulating levels of Leptin have been shown to be regulated in response to a variety of stimuli including food intake, insulin, glucocorticoids, cytokines, and reproductive events (2, 10-14). The majority of Leptin in serum was reported to be bound to multiple Leptin-binding proteins (15).

The Quantikine® Mouse/Rat Leptin Immunoassay is a 4.0 hour solid phase ELISA designed to measure mouse or rat Leptin in cell culture supernates, tissue homogenates, serum, and plasma. It contains *E. coli*-expressed recombinant mouse Leptin and antibodies raised against the recombinant factor. This immunoassay has been shown to quantitate the recombinant mouse Leptin accurately. Results obtained for naturally occurring mouse or rat Leptin 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 mouse or rat Leptin.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for mouse/rat Leptin has been pre-coated onto a microplate. Standards, control, and samples are pipetted into the wells and any Leptin present is bound by the immobilized antibody. After washing away any unbound substances, a biotinylated monoclonal antibody specific for mouse/rat Leptin is added to the wells. Following a wash to remove any unbound antibody-biotin reagent, an enzyme-linked streptavidin polymer is added to the wells. After washing away any unbound streptavidin polymer-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of Leptin 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 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.
- 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 #	CATALOG # MOB00B	CATALOG # SMOB00B	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Mouse/Rat Leptin Microplate	899157	1 plate	6 plates	96 well polystyrene microplates (12 strips of 8 wells) coated with a monoclonal antibody specific for mouse/rat Leptin.	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.*
Mouse/Rat Leptin Standard	899159	2 vials	12 vials	Recombinant mouse Leptin in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	Use a new standard and control for each assay. Discard after use.
Mouse/Rat Leptin Control	899160	2 vials	12 vials	Recombinant mouse Leptin in a buffered protein base with preservatives; lyophilized. The assay value of the control should be within the range specified on the label.	
Streptavidin-HRP 1	898508	1 vial	6 vials	12 mL of a solution with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Mouse/Rat Leptin Conjugate	899158	1 vial	6 vials	12 mL/vial of a monoclonal antibody specific for mouse/rat Leptin conjugated to biotin with preservatives.	
Assay Diluent RD1-55	895066	1 vial	3 vials	11 mL/vial of a buffered protein base with preservatives.	
Calibrator Diluent RD5-16	895302	1 vial	6 vials	21 mL/vial of a buffered protein base with preservatives.	
Wash Buffer Concentrate	895003	2 vials	12 vials	21 mL/vial of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Color Reagent A	895000	1 vial	3 vials	12 mL/vial of stabilized hydrogen peroxide.	
Color Reagent B	895001	1 vial	3 vials	12 mL/vial of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895174	1 vial	3 vials	23 mL/vial of diluted hydrochloric acid.	
Plate Sealers	N/A	8 strips	24 strips	Adhesive strips.	

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

MOB00B contains sufficient materials to run ELISAs on one 96 well plates.
SMOB00B (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems®, Catalog # PMOB00B). Refer to the PharmPak Contents section for specific vial counts.

PHARMPAK CONTENTS

Each PharmPak contains reagents sufficient for the assay of 50 microplates (96 wells/plate). The package inserts supplied are the same as those supplied in the single kit packs and because of this, a few minor differences related to the number of reagents and their container sizes should be noted.

- Sufficient material is supplied to perform at least 50 standard curves; reuse of each vial may be required. The number of vials, and the number of standard curves obtained per vial will vary with the analyte.
- Wash Buffer 25X Concentrate is bulk packed in 125 mL bottles containing 100 mL.
  Note: *Additional wash buffer is available for purchase (R&D Systems®, Catalog # WA126).*

The reagents provided in this PharmPak are detailed below.

PART	PART #	QUANTITY
Mouse/Rat Leptin Microplate	899157	50 plates
Mouse/Rat Leptin Conjugate	899158	50 vials
Mouse/Rat Leptin Standard	899159	25 vials
Mouse/Rat Leptin Control	899160	25 vials
Assay Diluent RD1-55	895066	25 vials
Streptavidin-HRP 1	898508	50 vials
Calibrator Diluent RD5-16	895302	50 vials
Color Reagent A	895000	25 vials
Color Reagent B	895001	25 vials
Wash Buffer Concentrate	895126	12 bottles
Stop Solution	895032	25 vials
Plate Sealers	N/A	200 sheets
Package Inserts	753307	2 booklets

PRECAUTIONS

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 SDS on our website prior to use.

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
- 1000 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

ADDITIONAL REAGENTS REQUIRED

Note: *For sample activation of pregnant mouse serum only (second trimester through post-partum Day 1)*

- Glacial acetic acid (Reagent Grade A.C.S., 17.4 N)
- HEPES, free acid (Reagent Grade M.W., 238.8)
- Sodium hydroxide (Reagent Grade A.C.S., 10 N)
- Urea (Reagent Grade M.W., 60.06)

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.

Tissue Homogenates - Prior to assay, tissues must be homogenized according to the directions in the Sample Values section.

Mouse Serum - Allow blood samples to clot for 2 hours at room temperature before centrifuging for 20 minutes at 2000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Rat Serum - Allow blood samples to clot for 2 hours at room temperature before centrifuging for 20 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Mouse Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 20 minutes at 2000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Rat Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 20 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.*

ACTIVATION REAGENT PREPARATION

To activate Leptin to the immunoreactive form (see Sample Preparation), prepare the following solutions for acid activation and neutralization. The solutions may be stored in polypropylene bottles at room temperature for up to one month. If any precipitation forms, gently heat the solution to 37 °C while mixing. **Caution:** *Wear protective clothing and safety glasses during preparation or use of these reagents.*

2.5 N Acetic Acid/8 M Urea (250 mL) - To 100 mL of deionized water, slowly add 35.9 mL of 17.4 N (Glacial) Acetic Acid. Mix well. Add 120.2 g Urea. Mix well until dissolved. Bring final volume to 250 mL with deionized water.

2.7 N NaOH/1 M HEPES (250 mL) - To 140 mL of deionized water, add 67.5 mL of 10 N NaOH. Mix well. Add 59.5 g HEPES. Mix well. Bring final volume to 250 mL with deionized water.

SAMPLE ACTIVATION PROTOCOL FOR PREGNANT MOUSE SERUM

Note: *Do not acid activate the Mouse/Rat Leptin Standards or Control. The kit standard and control contain immunoreactive mouse Leptin.*

1. To 10 µL serum, add 20 µL of 2.5 N Acetic Acid/8 M Urea.
2. Mix well.
3. Incubate 10 minutes at room temperature.
4. Neutralize the acidified sample by adding 20 µL of 2.7 N NaOH/1 M HEPES.
5. Mix well.
6. Prior to the assay, dilute the activated serum sample 100-fold with Calibrator Diluent RD5-16. A suggested 100-fold dilution is 10 µL of activated sample + 990 µL of Calibrator Diluent RD5-16.

The concentration read off the standard curve must be multiplied by the total dilution factor, 500.

For each new lot of acidification and neutralization reagents, measure the pH of several representative samples after neutralization to ensure that the pH is 7.2-7.6. Adjust the volume and corresponding dilution factor of the neutralization reagent as needed.

SAMPLE PREPARATION

Normal mouse serum, plasma, and serum from pregnant mice within the first trimester of pregnancy require a 20-fold dilution into Calibrator Diluent RD5-16 due to a matrix effect. A suggested 20-fold dilution is 10 µL of sample + 190 µL of Calibrator Diluent RD5-16.

Normal rat serum and plasma require a 2-fold dilution into Calibrator Diluent RD5-16 due to high endogenous levels. A suggested 2-fold dilution is 100 µL of sample + 100 µL of Calibrator Diluent RD5-16.

Pregnant mouse serum from the second trimester of pregnancy through post-partum day 1 require acid/urea activation of mouse Leptin. **Note:** *Pregnant rat serum has not been tested in this kit.*

Tissue homogenate samples can be tested neat or may require a dilution due to high endogenous levels. **Note:** *Quantitation of sample protein concentration using a total protein assay is recommended. The suggested range for total protein added is 3-25 µg/mL.*

Multiple dilutions are recommended for unknown samples.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

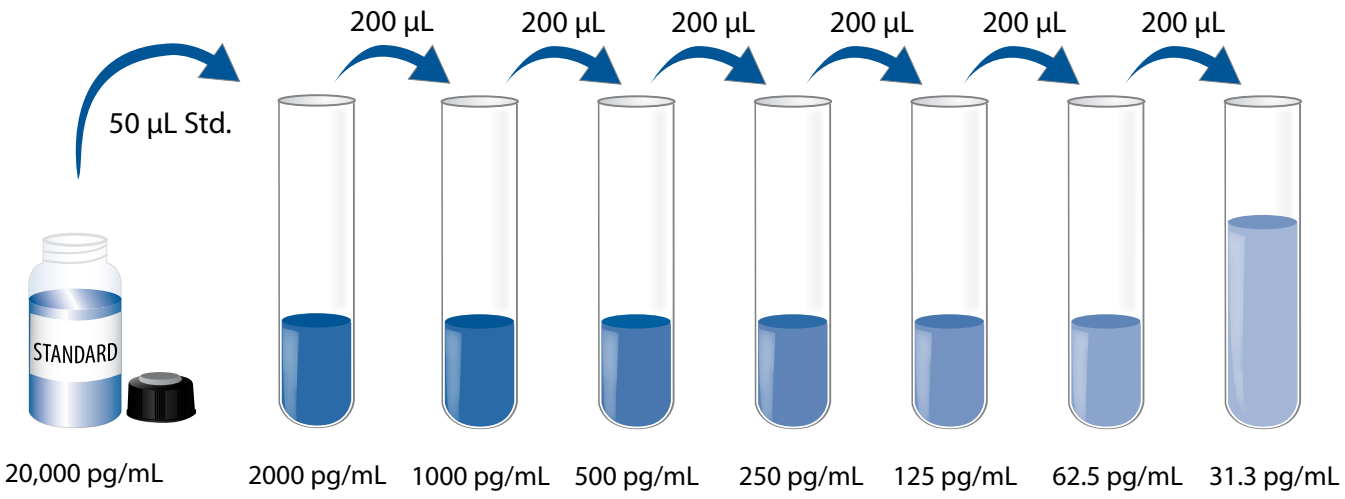
Mouse/Rat Leptin Control - Reconstitute the control with 1.0 mL deionized or distilled water. Mix thoroughly. Assay the control undiluted.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 40 mL of Wash Buffer Concentrate to 960 mL of deionized or distilled water to prepare 1000 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. 100 µL of the resultant mixture is required per well.

Mouse/Rat Leptin Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Mouse/Rat Leptin Standard with deionized or distilled water. This reconstitution produces a stock solution of 20,000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle mixing prior to making dilutions.

Pipette 450 µL of Calibrator Diluent RD5-16 into the 2000 pg/mL tube. Pipette 200 µL into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 2000 pg/mL standard serves as the high standard. Calibrator Diluent RD5-16 serves as the zero standard (0 pg/mL).



ASSAY PROCEDURE

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

1. Prepare reagents, samples, and working standards as directed by the previous section.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 50 µL of Assay Diluent RD1-55 to each well.
4. Add 50 µL of standard, control, or sample* per well. Cover with 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 100 µL of Mouse/Rat Leptin Conjugate per well. Cover with a new adhesive strip. Incubate for **1 hour** at room temperature on the shaker.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of Streptavidin-HRP 1 to each well. Cover with a new adhesive strip. Incubate for **30 minutes** at room temperature on the shaker.
9. Repeat the aspiration/wash as in step 5.
10. Add 100 µL of Substrate Solution to each well. Incubate for 30 minutes **on the benchtop** at room temperature. **Protect from light.**
11. Add 100 µL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
12. 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

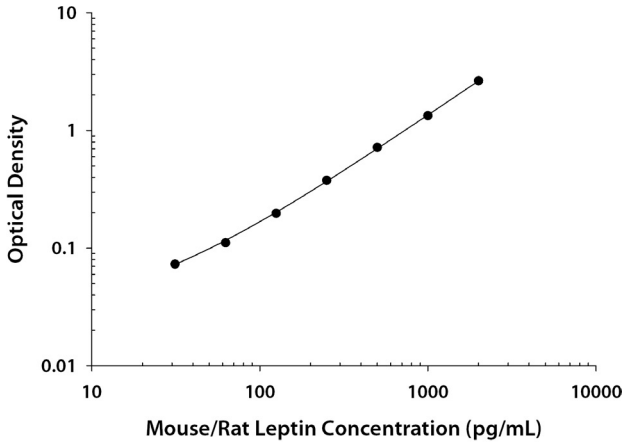
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

Create a standard curve by reducing the data using computer software capable of generating a log/log 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 a log/log graph. The data may be linearized by plotting the log of the mouse/rat Leptin concentrations versus the log of the O.D. on a linear scale, and the best fit line can be determined by regression analysis.

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

TYPICAL DATA

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(pg/mL)	O.D.	Average	Corrected
0	0.033 0.034	0.034	—
31.3	0.073 0.073	0.073	0.039
62.5	0.111 0.111	0.111	0.077
125	0.193 0.202	0.198	0.164
250	0.375 0.378	0.377	0.343
500	0.719 0.721	0.720	0.686
1000	1.336 1.340	1.338	1.304
2000	2.644 2.648	2.646	2.612

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 twenty separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of components.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (pg/mL)	152	421	1512	143	389	1469
Standard deviation	5.40	8.58	41.0	11.1	30.5	87.2
CV (%)	3.6	2.0	2.7	7.8	7.8	5.9

RECOVERY

The recovery of mouse/rat Leptin spiked to levels throughout the range of the assay in various matrices was evaluated.

Mouse Samples	Average % Recovery	Range
Cell culture media (n=4)	109	98-117%
Serum* (n=4)	95	90-102%
EDTA plasma* (n=4)	96	89-110%
Heparin plasma* (n=4)	90	80-105%

Rat Samples	Average % Recovery	Range
Serum* (n=4)	98	91-103%
EDTA plasma* (n=4)	106	99-113%
Heparin plasma* (n=4)	98	91-108%

*Samples may require dilution. See Sample Preparation Section.

SENSITIVITY

Twenty-five assays were evaluated and the minimum detectable dose (MDD) of mouse/rat Leptin ranged from 1.58-5.56 pg/mL. The mean MDD was 2.58 pg/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, four or more samples containing and/or spiked with high concentrations of mouse/rat Leptin in each matrix were diluted with calibrator diluent and assayed. Results from typical sample dilutions are shown.

Mouse Samples		Cell culture media (n=4)	Serum* (n=4)	Pregnant mouse serum* (n=4)	EDTA plasma* † (n=4)	Heparin plasma* (n=4)
1:2	Average % of Expected	95	108	111	112	108
	Range (%)	94-95	103-114	108-115	109-115	101-117
1:4	Average % of Expected	95	106	119	112	107
	Range (%)	95-95	99-116	116-123	106-117	99-114
1:8	Average % of Expected	93	103	120	110	103
	Range (%)	90-96	92-121	118-121	103-118	101-106
1:16	Average % of Expected	92	108	116	112	103
	Range (%)	88-95	97-123	110-121	107-119	96-110

Rat Samples		Cell culture media (n=4)	Serum (n=4)	EDTA plasma* (n=4)	Heparin plasma (n=4)
1:2	Average % of Expected	95	107	103	110
	Range (%)	94-95	106-108	100-106	109-112
1:4	Average % of Expected	95	114	105	114
	Range (%)	95-95	110-118	97-112	112-118
1:8	Average % of Expected	93	115	103	111
	Range (%)	90-96	110-122	97-110	109-113
1:16	Average % of Expected	92	115	102	114
	Range (%)	88-95	110-121	96-109	109-119

* Samples were diluted prior to assay.

† Samples were activated using activation procedure.

CALIBRATION

This immunoassay is calibrated against a highly purified *E. coli*-expressed recombinant mouse Leptin produced at R&D Systems®.

The NIBSC/WHO Mouse Leptin (mouse rDNA-derived) International Standard 97/626 was evaluated in this kit. The dose response curve of the International Standard 97/626 parallels the Quantikine® standard curve. To convert sample values obtained with the Quantikine® Mouse/Rat Leptin kit to approximate NIBSC/WHO 97/626 Units, use the equation below.

NIBSC/WHO (97/626) approximate value (mIU/mL) = 0.7836 x Quantikine® Mouse/Rat Leptin value (pg/mL)

Note: *Based on data generated in December 2019.*

SAMPLE VALUES

Serum/Plasma - Samples were evaluated for the presence of mouse/rat Leptin in this assay.

Sample		Mean (pg/mL)	Range (pg/mL)	Standard Deviation (pg/mL)
Mouse serum (NSA mice, 6 weeks)	(n=4)	7766	2049-12,784	4574
Mouse serum (Pregnant mice, NSA)*	(n=4)	430,296	292,580-670,000	176,207
Mouse serum (CD-1 females)	(n=10)	3037	1047-6951	2106
Mouse serum (Retired breeders)	(n=16)	14,245	3038-31,451	8914
Mouse EDTA plasma (Retired breeders)	(n=8)	20,723	5465-23,708	7212
Mouse heparin plasma (Retired breeders)	(n=8)	19,794	5625-37,997	11,216
Rat serum	male (n=5)	1275	950-1550	221
	female (n=5)	728	401-892	197
Rat EDTA plasma	male (n=5)	1725	1034-2463	536
	female (n=5)	1305	1091-1591	180
Rat heparin plasma	male (n=5)	1457	1206-1650	212
	female (n=5)	1311	562-1927	490

*Samples were activated using activation procedure.

Cell Culture Supernates - 3T3-L1 mouse embryonic fibroblast adipose-like cells were left untreated or differentiated into adipocytes by treating with 115 µg/mL IBMX, 10 µg/mL insulin, and 390 ng/mL dexamethasone for 48 hours, followed by the addition of 10 µg/mL insulin for another 48 hours. Regular media was then added for every 48 hours for 96 additional hours. Aliquots of the cell culture supernates were removed and assayed for levels of mouse Leptin.

Condition	(pg/mL)
Untreated	ND
Differentiated	6918

ND=Non-detectable

Tissue Hemogenates:

NSA Mouse Adipose tissue was collected from 10-15 mice and homogenized in PBS. The homogenized material was then freeze-thawed for three cycles at ≤ -80 °C and then centrifuged at 2-8 °C for 15 minutes at 3000 x g. An aliquot of the homogenate was removed, assayed for mouse Leptin, and measured 637 pg/mL.

Sprague Dawley Rat Adipose Tissue was collected from 1 rat and homogenized in PBS. The homogenized material was then freeze-thawed for three cycles at ≤ -20 °C and then centrifuged at 2-8 °C for 20 minutes at 3000 x g. An aliquot of the homogenate was removed, assayed for rat Leptin, and measured 186 pg/mL.

SPECIFICITY

This assay recognizes natural and recombinant mouse and rat Leptin.

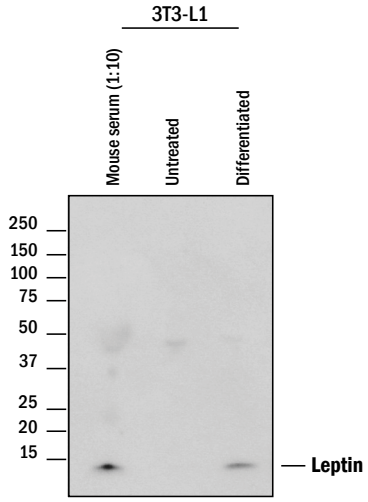
The factors listed below were prepared at 50 ng/mL in calibrator diluent and assayed for cross-reactivity. Preparations of the following factors prepared at 50 ng/mL in a mid-range mouse Leptin control were assayed for interference. No significant cross-reactivity or interference was observed.

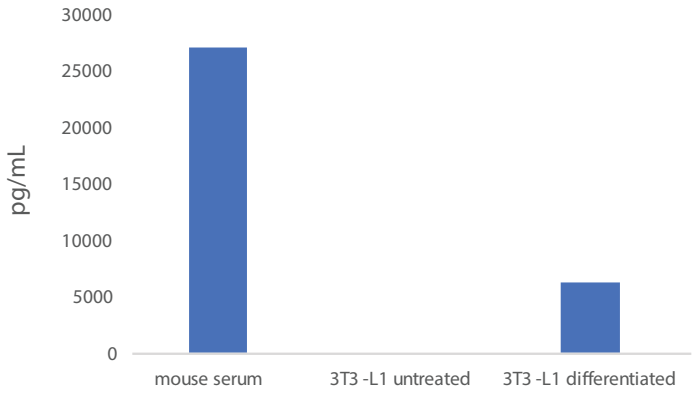
Recombinant mouse:

C10
G-CSF
G-CSF R
GM-CSF
gp130
IFN-γ
IL-1α
IL-1β
IL-2
IL-3
IL-4
IL-5
IL-6
IL-7
IL-9
IL-10
IL-10 R
IL-10 Rα
IL-10 Rβ
IL-11
IL-12
IL-13
JE/MCP-1
Leptin R
LIF
LIF R
M-CSF
MIP-1α
MIP-1β
MIP-2
SCF
TNF-α
Tpo R
VEGF
$VEGF_{164}$

Recombinant human Leptin cross-reacts approximately 0.09% and interferes at concentrations > 10 ng/mL.

Recombinant mouse Leptin R does not cross-react but does interfere at concentrations > 2000 pg/mL in this assay.





Conditioned media samples were analyzed by Western Blot and Quantikine® ELISA. 3T3-L1 cells were left untreated, or differentiated into adipocytes by treating with 115 µg/mL IBMX, 10 µg/mL insulin, and 390 ng/mL dexamethasone for 48 hours, followed by the addition of 10 µg/mL insulin for another 48 hours. Regular media was then added every 48 hours for 96 additional hours. For Western Blot, mouse serum was diluted 1:10, and 3T3-L1 samples were run neat. Western samples were resolved under reducing SDS-PAGE conditions, transferred to PVDF membrane, and immunoblotted with goat anti-mouse Leptin/OB (R&D Systems®, Catalog # AF498). The Western Blot shows a direct correlation with ELISA values for these samples.

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