Performance characteristics, continued

Intra-assay precision

Samples of known Ms leptin concentration were assayed in replicates of 16 to determine precision within an assay.

Parameters	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	2011.38	653.54	175.24
SD	48.53	31.6	14.86
%CV	2.41	4.84	8.48

SD = Standard Deviation; CV = Coefficient of Variation

Inter-assay precision

Samples were assayed 48 times in multiple assays to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	2046.77	661.19	185.53
SD	75.89	30.69	18.35
%CV	3.71	4.64	9.89

SD = Standard Deviation; CV = Coefficient of Variation

Recovery

The recovery of recombinant Ms leptin added to mouse serum, EDTA plasma, citrate plasma, heparin plasma, and tissue culture medium containing 10% fetal bovine serum was measured on the Ms Leptin ELISA.

Sample	Average % Recovery
Serum*	108.1
EDTA plasma*	102.7
Citrate plasma*	97.9
Heparin plasma*	93.9
DMEM + 10% fetal bovine serum	101.5
RPMI+10% fetal bovine serum	95.8

* Due to high endogenous levels of Ms leptin, serum and plasma were prediluted 10-fold prior to spiking with recombinant Ms Leptin.

Linearity of dilution

Mouse serum, EDTA plasma, citrate plasma, heparin plasma, and tissue culture medium spiked with recombinant Ms Leptin were serially diluted in Standard Diluent Buffer over the range of the assay. Linear regression analysis of samples versus the expected concentration yielded the following correlation coefficients.

Sample	Correlation Coefficient
Serum	0.996
EDTA plasma	0.984
Citrate plasma	0.993
Heparin plasma	0.959
Tissue culture media	1.0

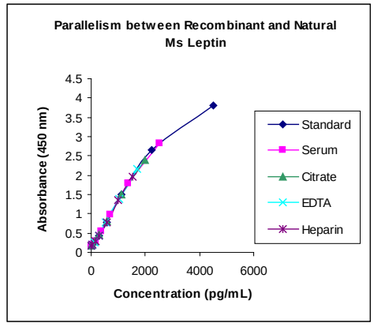
Expected values

Random serum and plasma samples (from normal mice, as well as those at various stages of pregnancy) were evaluated for the presence of Ms leptin in this assay.

Sample	Range (pg/mL)	Average (pg/mL)
Serum (n=19)	666–5912	3366
EDTA plasma (n=4)	1863–3661	2936
Citrate plasma (n=4)	2042–2973	2674
Heparin plasma (n=4)	2483–4357	3388
Early stage pregnant serum (n=3)	3559–11158	7229
Mid stage pregnant serum (n=2)	9862–12292	11077
Late stage pregnant serum (n=3)	90474–241504	174954

Parallelism

Random mouse serum and plasma samples were serially diluted in Standard Diluent Buffer. The optical density of each dilution was plotted against the Ms Leptin standard curve. Parallelism was demonstrated and indicated that the standard accurately reflects the Ms leptin content in natural samples.



Important licensing information

This product may be covered by one or more Limited Use Label Licenses. By use of this product, you accept the terms and conditions of all applicable Limited Use Label Licenses.

Limited product warranty

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Product label explanation of symbols and warnings

REF	Catalog Number	LOT	Batch code		Temperature limitation		Use by		Manufacturer		Consult instructions for use		Caution, consult accompanying documents

For support visit www.lifetechnologies.com/support or contact **techsupport@lifetech.com**.

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Mouse Leptin ELISA Kit

Catalog. no. KMC2281 **Quantity:** 96 tests

Pub. No. MAN0004050 **Rev** 1.0

Description

The Mouse Leptin ELISA Kit is a solid-phase sandwich Enzyme Linked Immunosorbent Assay (ELISA). This assay is designed to detect and quantify the level of mouse leptin in serum, plasma, buffered solution, and cell culture supernatants. The assay will recognize both natural and recombinant mouse leptin.

Leptin is a peptide hormone that plays a key role in regulating appetite and adiposity. It is produced primarily by adipose tissue, and plasma leptin concentrations correlate with adiposity. Other sources of leptin include placenta, pituitary, and central nervous system. Plasma levels are generally higher in females than in males. An age-dependent diurnal pattern of expression has been observed in both sexes. Stimuli that reduce leptin plasma concentrations include cold temperature and catecholamines.

Leptin, shares homology with the cytokine interleukin-6. Mouse leptin is 96% and 84% homologous with the rat and human proteins, respectively.

Contents and storage

The components included in the ELISA kit are listed below. Upon receipt, store the kit at 2°C to 8°C.

Components	96 Test Kit
Ms Leptin Antibody Coated Wells. 96 well plate.	1 plate
Ms Leptin Biotin Conjugate. Contains 0.1% sodium azide.	11 mL
Ms Leptin Standard. Lyophilized. Contains 0.1% sodium azide. Refer to vial label for quantity and reconstitution volume.	2 vials
Wash Buffer Concentrate (25X).	100 mL
Standard Diluent Buffer. Contains 0.1% sodium azide.	60 mL
Streptavidin-HRP (100X). Contains 3.3 mM thymol.	0.125 mL
Streptavidin-HRP Diluent. Contains 3.3 mM thymol.	25 mL
Stabilized Chromogen, Tetramethylbenzidine (TMB).	25 mL
Stop Solution.	25 mL
Adhesive Plate Covers.	4

CAUTION! This kit contains materials with small quantities of sodium azide. Sodium azide reacts with lead and copper plumbing to form explosive metal azides. Upon disposal, flush drains with a large volume of water to prevent azide accumulation. Avoid ingestion and contact with eyes, skin and mucous membranes. In case of contact, rinse affected area with plenty of water. Observe all federal, state, and local regulations for disposal.

Materials required but not provided

- Distilled or deionized water
- Microtiter plate reader with software capable of measurement at or near 450 nm
- Plate washer–automated or manual (squirt bottle, manifold dispenser, or equivalent)
- Calibrated adjustable precision pipettes and glass or plastic tubes for diluting solutions

General Guidelines

- Review the **Procedural guidelines** and **Plate washing directions** in the *ELISA Technical Guide* available at **www.lifetechnologies.com/manuals** for details prior to starting the procedure.
- Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.

For Research Use Only. Not for use in diagnostic procedures.

Manufacturing Site • 7335 Executive Way • Frederick • MD 21704 • E-mail: **techsupport@lifetech.com**

Prepare 1X Wash Buffer

1. Allow the Wash Buffer Concentrate (25X) to reach room temperature and mix to redissolve any precipitated salts.
2. Dilute 16 mL of Wash Buffer Concentrate (25X) with 384 mL of deionized or distilled water. Label as 1X Wash Buffer.
3. Store the concentrate and 1X Wash Buffer in the refrigerator. Use the diluted buffer within 14 days.

Sample preparation guidelines

- Collect samples in pyrogen/endotoxin-free tubes.
- Freeze samples after collection if samples will not be tested immediately. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well (do not vortex) prior to analysis.
- Avoid the use of hemolyzed or lipemic sera. If large amounts of particulate matter are present in the sample, centrifuge or filter sample prior to analysis.

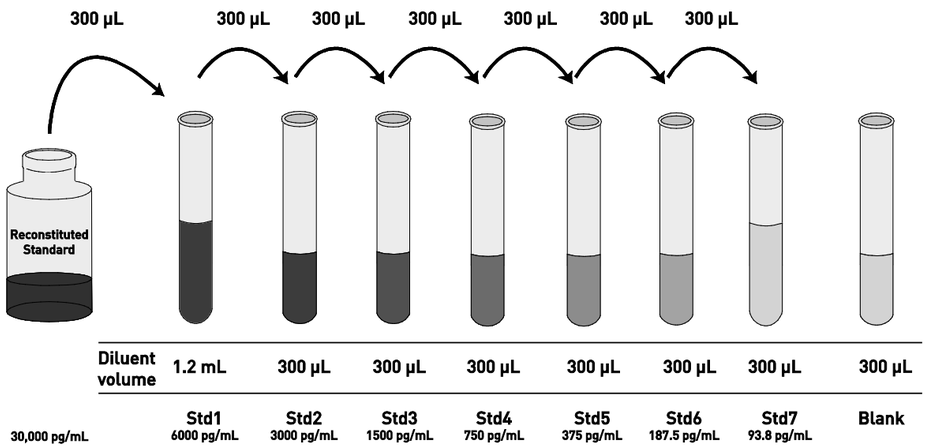
Dilute samples

- Dilute **serum and plasma** samples 5-fold in Standard Diluent Buffer.
- Assay **cell culture supernatant** samples neat.
- Serum, plasma, or tissue culture supernatant samples that are >6000 pg/mL should be diluted in their respective buffers.
- Because conditions may vary, it is recommended that each investigator determine the optimal dilution to be used for each application.

Dilute standards

Note: Use glass or plastic tubes for diluting standards.
This assay has been calibrated against the WHO reference preparation 97/626 (NIBSC, Hertfordshire, UK, EN6 3QG).

1. Reconstitute Ms Leptin Standard to 30,000 pg/mL with Standard Diluent Buffer. Refer to the standard vial label for instructions. Swirl or mix gently and allow the contents to sit for 10 minutes to ensure complete reconstitution. Label as 30,000 pg/mL Ms Leptin. **Use the standard within 15 minutes of reconstitution**.
2. Add 300 µL Reconstituted Standard to one tube containing 1.2 mL Standard Diluent Buffer and label as 6000 pg/mL Ms Leptin.
3. Add 300 µL Standard Diluent Buffer to each of 7 tubes labeled as follows: 3000, 1500, 750, 375, 187.5, 93.8, and 0 pg/mL Ms Leptin.
4. Make serial dilutions of the standard as described below in the dilution diagram. Mix thoroughly between steps.
5. Discard any remaining reconstituted standard. Return the Standard Diluent Buffer to the refrigerator.



Prepare Streptavidin-HRP solution

Note: Prepare Streptavidin-HRP within 15 minutes of usage.

The Streptavidin-HRP (100X) is in 50% glycerol, which is viscous. To ensure accurate dilution:

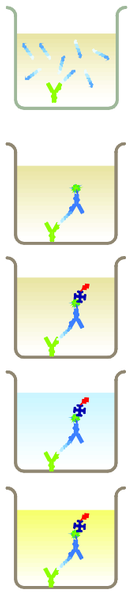
1. For each 8-well strip used in the assay, pipet 10 µL Streptavidin-HRP (100X) solution, wipe the pipette tip with a clean absorbent paper to remove any excess solution, and dispense the solution into a tube containing 1 mL of HRP Diluent. Mix thoroughly.
2. Return the unused Streptavidin-HRP (100X) solution to the refrigerator.

ELISA procedure

Allow all reagents to reach room temperature before use. Mix all liquid reagents prior to use. **Total assay time is 4 hours.**

IMPORTANT! Perform a standard curve with each assay.

Determine the number of 8-well strips required for the assay. Insert the strips in the frames for use. Re-bag any unused strips and frames, and store at 2 to 8°C for future use.



Bind antigen

1. Add 100 µL of standards, background controls, or samples (see page 2) to the appropriate wells. Leave wells for chromogen blanks empty.
2. Cover the plate with plate cover and incubate for 2 hours at 37°C.
3. Thoroughly aspirate the solution and wash wells 4 times with 1X Wash Buffer.

Add detector antibody

4. Add 100 µL Ms Leptin Biotin Conjugate solution into each well except the chromogen blanks.
5. Cover the plate with plate cover and incubate for 1 hour at room temperature.
6. Thoroughly aspirate the solution and wash wells 4 times with 1X Wash Buffer.

Add Streptavidin-HRP

7. Add 100 µL Streptavidin-HRP (see page 2) into each well except the chromogen blanks.
8. Cover the plate with plate cover and incubate for 30 minutes at room temperature.
9. Thoroughly aspirate the solution from the wells and wash wells 5 times with 1X Wash Buffer.

Add chromogen

10. Add 100 µL Stabilized Chromogen to each well. The substrate solution will begin to turn blue.
11. Cover the plate with plate cover and incubate for 30 minutes at room temperature **in the dark**.
    Note: TMB should not touch aluminum foil or other metals.

Add stop solution

12. Add 100 µL Stop Solution to each well. Tap side of the plate gently to mix. The solution in the wells changes from blue to yellow.

Read the plate and generate the standard curve

1. Read the absorbance at 450 nm. Read the plate within 2 hours after adding the Stop Solution.
2. Use curve-fitting software to generate the standard curve. A four parameter algorithm provides the best standard curve fit. Optimally, the background absorbance may be subtracted from all data points, including standards, unknowns and controls, prior to plotting.
3. Read the concentrations for unknown samples and controls from the standard curve. Multiply value(s) obtained for sample(s) by the appropriate factor to correct for the sample dilution.
   Note: Dilute samples producing signals greater than that of the highest standard in Standard Diluent Buffer and reanalyze. Multiply the concentration by the appropriate dilution factor.

Performance characteristics

Standard curve (example)

The following data were obtained for the various standards over the range of 0–6000 pg/mL Ms leptin.

Standard Ms Leptin (pg/mL)	Optical Density (450 nm)
6000	3.238
3000	2.063
1500	1.175
750	0.683
375	0.437
187.5	0.320
93.8	0.265
0	0.195

Specificity

Buffered solutions of a panel of substances ranging in concentration from 400–70,000 pg/mL were assayed with the Ms Leptin kit and found to have no cross-reactivity: **Human** insulin, adiponectin; **Mouse** FGFb, GM-CSF, IFN-γ, IL-1α, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12, IL-13, IL-17, IP-10, KC, MCP-1, MIG, MIP-1α, TNF-α, VEGF. Cross-reactivity was observed with recombinant rat leptin, recombinant human leptin, and recombinant ovine leptin (76%, 17.3%, and 3.5%, respectively). Random, normal serum samples were also evaluated with the kit. No cross-reactivity was observed with bovine or rabbit serum. There was low cross-reactivity with hamster and rat serum, moderate cross-reactivity with human serum, and significant cross-reactivity with monkey, swine and goat serum.

Sensitivity

The minimum detectable dose of Ms leptin is <50 pg/mL. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 30 times, and calculating the corresponding concentration.