Lep (Mouse/Rat) ELISA Kit

Catalog Number KA0026

96 assays

Version: 08

Intended for research use only

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Introduction

Intended Use

The Lep (Mouse/Rat) ELISA Kit is a sandwich enzyme immunoassay for the quantitative measurement of mouse and/or rat leptin.

Features

- ✓ It is intended for research use only.
- ✓ The total assay time is less than 3.5 hours.
- ✓ The kit measures leptin in serum and plasma (EDTA, citrate, heparin).
- ✓ Assay format is 96 wells.
- ✓ Quality Controls are mouse and rat serum based. No human sera are used.
- ✓ Standards are recombinant protein based.
- ✓ Components of the kit are provided ready to use, concentrated or lyophilized.

Background

Leptin is a protein hormone with important effects in metabolism and regulating body weight. It is a single-chain 16 kDa protein consisting of 146 amino acid residues and encoded by the obese (ob) gene.
Leptin is expressed predominantly by adipocytes, small amounts of leptin are also secreted by cells in the epithelium of stomach and in the placenta. Leptin´s effect on body weight is mediated through effects on hypothalamic centers, where leptin receptors are highly expressed. Leptin has a dual action, it decreases the appetite and increases energy consumption.
A mutations in the ob gene of leptin or in the gene of leptin receptor causes hyperphagia, reduced energy expenditure, and severe obesity in the *ob/ob* mice.
Ob gene knockout mice are also characterized by several metabolic abnormalities including hyperglucocorticoidemia, hyperglycaemia, hyperinsulinemia and insulin resistance.
When *ob/ob* mice are treated with injections of leptin, they lose their excess fat and return to normal body weight.
Studies have shown that leptin appears to be a significant regulator of reproductive function. In addition, leptin is involved in bone metabolism and plays a significant role as an immunomodulator.

Areas of investigation:
Energy metabolism and body weight regulation

Principle of the Assay

In the Lep (Mouse/Rat) ELISA Kit, standards, quality controls and samples are incubated in microplate wells pre-coated with anti-mouse leptin antibody. After 60 minutes incubation and washing, biotin labelled polyclonal anti-mouse leptin antibody is added to the wells and incubated with the captured leptin for 60 minutes. After another washing, streptavidin-HRP conjugate is added. After 30 minutes incubation and the last washing step, the remaining 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. 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	Amount
Antibody Coated Microtiter Strips (Ready to use)	96 wells
Biotin Labelled Antibody Conc.(10x)	1.3 mL
Streptavidin-HRP Conjugate (Ready to use)	13 mL
Master Standard Mouse (lyophilized)	2 vials
Master Standard Rat (lyophilized)	2 vials
Quality Control Mouse (lyophilized)	2 vials
Quality Control Rat (lyophilized)	2 vials
Dilution Buffer (Ready to use)	13 mL x 2
Biotin–Ab Diluent (Ready to use)	13 mL
Wash Solution Conc. (10x)	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 5-1000 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

✓ Wear gloves and laboratory coats when handling experimental materials.

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

✓ 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.
- ✓ Use Mouse Leptin Standard to quantify leptin concentration in mouse samples.
- ✓ Use Rat Leptin Standard to quantify leptin concentration in rat samples

- 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.

- ✓ Streptavidin-HRP Conjugate
- ✓ Dilution Buffer
- ✓ Biotin-Ab Diluent
- ✓ 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:

- ✓ Mouse Leptin Standard

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

  Reconstitute the lyophilized Master Standard with Dilution Buffer just prior to the assay. Let it dissolve at least 15 minutes with occasional gentle shaking (not to foam). The resulting concentration of the mouse leptin in the stock solution is 4000 pg/mL (= M-Std. 4000 pg/mL).

  Prepare set of Mouse leptin standards using Dilution Buffer as follows:

Volume of Standard	Dilution Buffer	Concentration
M-Std. 4000 pg/mL	-	4000 pg/mL
250 µL M-Std. 4000 pg/mL	250 µL	2000 pg/mL
250 µL M-Std. 2000 pg/mL	250 µL	1000 pg/mL
200 µL M-Std. 1000 pg/mL	300 µL	400 pg/mL
250 µL M-Std. 400 pg/mL	250 µL	200 pg/mL
250 µL M-Std. 200 pg/mL	250 µL	100 pg/mL

  Prepared Standards are ready to use, do not dilute them.

✓ Rat Leptin Standard

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

Reconstitute the lyophilized Master Standard with Dilution Buffer just prior to the assay. Let it dissolve at least 15 minutes with occasional gentle shaking (not to foam). The resulting concentration of the rat leptin in the stock solution is 4000 pg/mL (= R-Std. 4000 pg/mL).

Prepare set of Rat leptin standards using Dilution Buffer as follow:

Volume of standard	Dilution Buffer	Concentration
R-Std. 4000 pg/mL	-	4000 pg/mL
250 µL R-Std. 4000 pg/mL	250 µL	2000 pg/mL
250 µL R-Std. 2000 pg/mL	250 µL	1000 pg/mL
200 µL R-Std. 1000 pg/mL	300 µL	400 pg/mL
250 µL R-Std. 400 pg/mL	250 µL	200 pg/mL
250 µL R-Std. 200 pg/mL	250 µL	100 pg/mL

Prepared Standards are ready to use, do not dilute them.

Stability and storage: Reconstituted Master Standard (4000 pg/mL) must be used immediately or aliquoted and frozen at -20°C for 3 months. Avoid repeated freeze/thaw cycles.

Do not store the diluted Standard solutions.

✓ Quality Controls Mouse / Rat

Refer to the Certificate of Analysis for current volume of Dilution Buffer needed for reconstitution and for current Quality Control concentration!!!

Reconstitute appropriate Quality Control (Mouse or Rat) with Dilution Buffer just prior to the assay. Let it dissolve at least 15 minutes with occasional gentle shaking (not to foam).

Reconstituted Quality Controls are ready to use, do not dilute them.

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

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

✓ Biotin Labelled Antibody Conc. (10x)

Prepare the working Biotin Labelled Antibody solution by adding 1 part Biotin Labelled Antibody Concentrate (10x) with 9 parts Biotin-Ab Diluent. Example: 100 µL of Biotin Labelled Antibody Concentrate (10x) + 900 µL of Biotin-Ab Diluent for 1 strip (8 wells).

Stability and storage: Opened Biotin Labelled Antibody Concentrate (10x) is stable 3 months when stored

at 2-8°C. Do not store the diluted Biotin Labelled Antibody solution.

- ✓ Wash Solution Conc. (10x)
  Dilute Wash Solution Concentrate (10x) ten-fold in distilled water to prepare a 1x working solution. Example: 100 mL of Wash Solution Concentrate (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 Concentrate (10x) is stable 3 months when stored at 2-8°C.

Sample Preparation

The kit measures leptin in serum and plasma (EDTA, citrate, 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.

Sample dilution:
Leptin levels may depend on feeding, diurnal cycle, pathophysiology, and strain of the animals. Suitable sample dilution should be tested by the researcher in advance. It is recommended to run 2-3 samples with various dilutions, e.g. 3x, 10x and 20x prior to the batch measurement to choose a suitable dilution for all the samples.

Dilution 3x
Dilute samples 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.

Dilution 10x
Dilute samples with the Dilution Buffer just prior to the assay, e.g. 15 µL of sample + 135 µL of Dilution Buffer for singlets, or preferably 30 µL of sample + 270 µL of Dilution Buffer for duplicates. Mix well (not to foam). Vortex is recommended.

Dilution 20x
Dilute samples 20x with the Dilution Buffer just prior to the assay, e.g. 7 µL of sample + 133 µL of Dilution Buffer for singlets, or preferably 14 µL of sample+ 266 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.

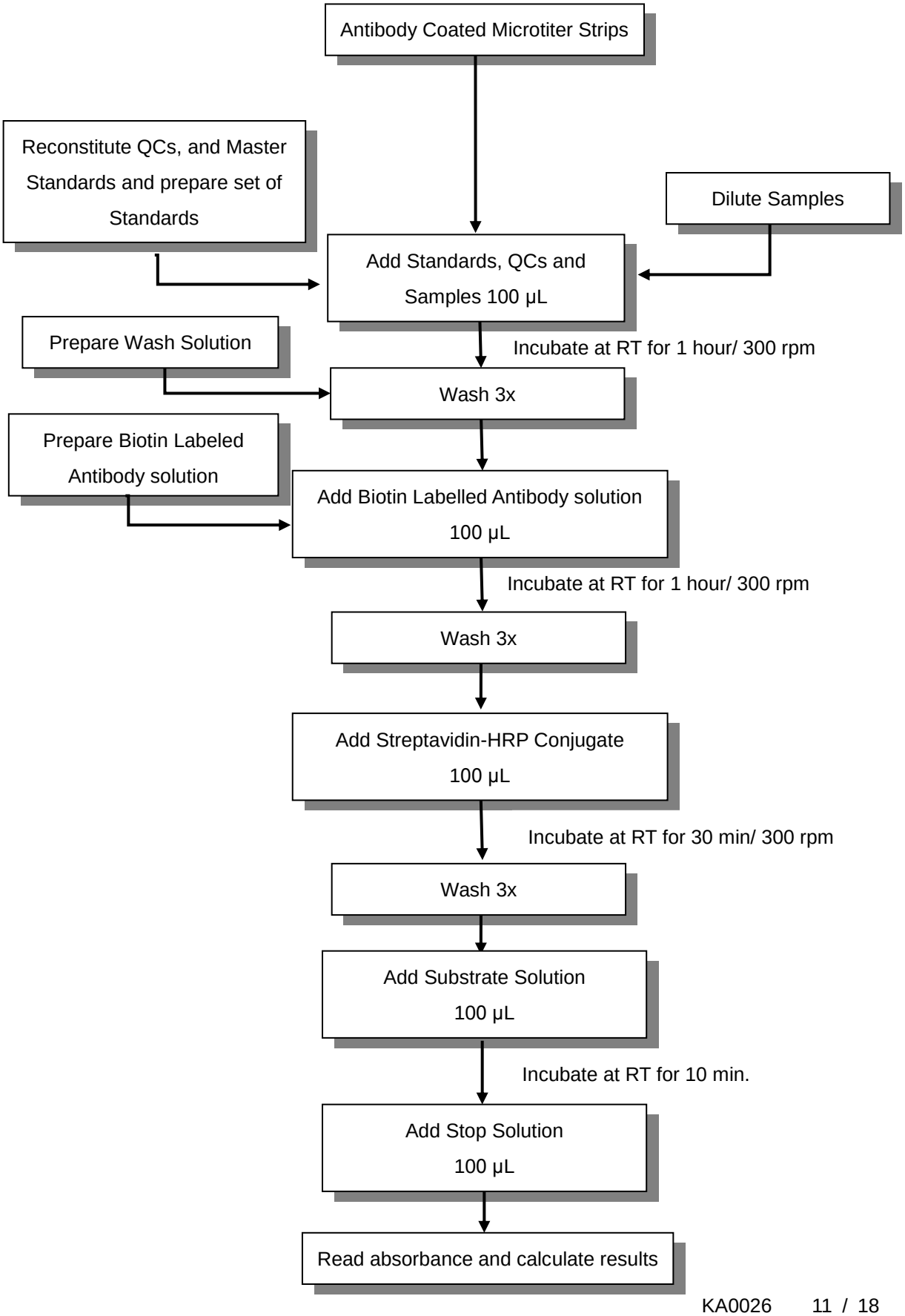
Assay Procedure

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 3-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 Biotin Labelled Antibody 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 3-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 Streptavidin-HRP Conjugate into each well.
8. Incubate the plate at room temperature (ca. 25°C) for 30 min., shaking at ca. 300 rpm on an orbital microplate shaker.
9. Wash the wells 3-times with Wash Solution (0.35 mL per well). After final wash, invert and tap the plate strongly against paper towel.
10. Add 100 µL of Substrate Solution into each well. Avoid exposing the microtiter plate to direct sunlight. Covering the plate with e.g. aluminium foil is recommended.
11. 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.
12. Stop the colour development by adding 100 µL of Stop Solution.
13. 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 12.

    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 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 (pg/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 samples calculated from the standard curve must be multiplied by their respective dilution factor, because samples have been diluted prior to the assay.

Example for 20x diluted samples: 500 pg/mL (from standard curve) x 20 (dilution factor) = 10000 pg/mL = 10 ng/mL.

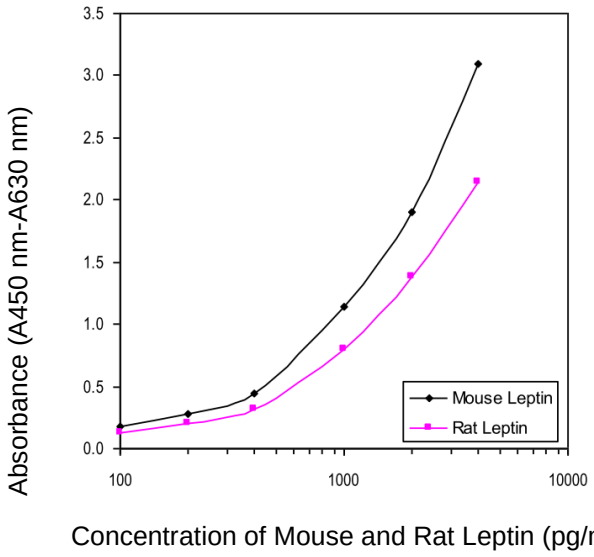


Figure 1: Typical Standard Curve for Lep (Mouse/Rat) ELISA Kit.

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 values in wells and is 30 pg/mL for mouse leptin and 50 pg/mL for rat leptin.

  *Dilution Buffer is pipetted into blank wells.

- Limit of assay

  Results exceeding leptin level of 4000 pg/mL should be repeated with more diluted samples (e.g 40x). Dilution factor needs to be taken into consideration in calculating the leptin concentration.

- Specificity

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

Mammalian serum sample	Observed cross reactivity
Bovine	no
Cat	no
Dog	no
Goat	no
Hamster	no
Horse	no
Human	yes
Monkey	no
Mouse	yes
Pig	no
Rabbit	no
Rat	yes

- Precision

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

Sample	Mean (ng/mL)	SD (ng/mL)	CV (%)
1 mouse	12.31	0.25	2.0
2 mouse	31.48	0.91	2.9
3 rat	9.74	0.18	1.8
4 rat	39.96	0.75	1.9

  Inter assay (Run-to-Run) (n=6)

Sample	Mean (ng/mL)	SD (ng/mL)	CV (%)
1 mouse	21.32	0.48	2.3
2 rat	17.13	0.76	4.4

- Spiking Recovery

  Serum samples were spiked with different amounts of mouse or rat leptin and assayed.

Sample	Observed (ng/mL)	Expected (ng/mL)	Recovery O/E (%)
1 mouse	12.02	-	-
	15.19	16.02	94.8
	18.46	20.02	92.2
	25.90	28.02	92.4
2 mouse	19.56	-	-
	21.86	23.56	92.8
	24.04	27.56	87.2
	32.03	35.56	90.8
3 rat	9.32	-	-
	13.33	13.32	100.1
	16.29	17.32	94.1
	25.47	25.32	100.6
4 rat	19.61	-	-
	22.41	23.61	94.9
	25.25	27.61	95.1
	34.83	35.61	97.8

- Linearity

  Serum samples were serially diluted with Dilution Buffer and assayed.

Sample	Dilution	Observed (ng/mL)	Expected (ng/mL)	Recovery O/E (%)
1 mouse	-	34.77	-	-
	2x	16.78	17.39	96.5
	4x	8.35	8.69	96.0
	8x	4.06	4.35	93.4
2 mouse	-	23.44	-	-
	2x	11.69	11.72	99.7
	4x	5.85	5.86	99.8
	8x	2.77	2.93	94.6
3 rat	-	29.67	-	-
	2x	14.53	14.83	98.0
	4x	7.11	7.42	95.8
	8x	3.91	3.71	105.5
4 rat	-	40.78	-	-
	2x	19.86	20.39	97.4
	4x	9.84	10.19	96.6
	8x	5.23	5.10	102.7

- Definition of the standard

  Mouse and Rat leptin standards are recombinant *E.coli* expressed proteins.

  Mouse leptin standard concentration was determined using the international standard: Leptin, mouse rDNA-derived 1st International Standard NIBSC code 97/626, Version 02, dated April 19 2004.

  No rat leptin international standard is available.

- Method Comparison

  The Lep (Mouse/Rat) ELISA Kit was not 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 4000	Standard 4000										
B	Standard 2000	Standard 2000										
C	Standard 1000	Standard 1000										
D	Standard 400	Standard 400										
E	Standard 200	Standard 200										
F	Standard 100	Standard 100										
G	Quality Control	Quality Control										
H	Blank	Blank										