Rat Leptin ELISA Kit

Catalog Number KRC2281 (96 tests)

Pub. No. MAN0004044 Rev. D.0 (34)

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.

Note: For safety and biohazard guidelines, see the "Safety" appendix in the *ELISA Technical Guide* (Pub. no. MAN0006706). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Product description

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

Leptin is a peptide hormone that plays a key role in regulating appetite and adiposity. It is produced primarily by adipose tissue with plasma leptin concentrations correlating with adiposity. Plasma levels are generally higher in females than in males with an age-dependent diurnal pattern of expression observed in both sexes. Stimuli that reduce leptin plasma concentrations include cold temperature and catecholamines. Leptin shares homology with the interleukin-6 cytokine. Rat leptin is 96% and 82% homologous with the mouse and human proteins, respectively.

Contents and storage

Upon receipt, store the kit at 2–8°C.

Contents	Cat. No. KRC2281 (96 tests)
Rt Leptin Standard, lyophilized; contains 0.1% sodium azide. Refer to vial label for quantity and reconstitution volume	2 vials
Standard Diluent Buffer; contains 0.1% sodium azide	25 mL
Rt Leptin Antibody Coated Plate, 96-well strip-well plate	1 plate
Rt Leptin Biotin Conjugate; contains 0.1% sodium azide	11 mL
Streptavidin-HRP (100X)	0.125 mL
Streptavidin-HRP Diluent; contains 3.3 mM thymol	25 mL
Wash Buffer Concentrate (25X)	100 mL
Stabilized Chromogen, Tetramethylbenzidine (TMB)	25 mL
Stop Solution	25 mL
Adhesive Plate Covers	4

Materials required but not supplied

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

Before you begin

IMPORTANT! Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.

- Review the **Procedural guidelines** and **Plate washing directions** in the *ELISA Technical Guide* available at **thermofisher.com**.
- Allow reagents to reach room temperature before use. Mix to redissolve any precipitated salts.

Prepare 1X Wash Buffer

1. Dilute 16 mL of Wash Buffer Concentrate (25X) with 384 mL of deionized or distilled water. Label as 1X Wash Buffer.
2. Store the concentrate and 1X Wash Buffer in the refrigerator. Use the diluted buffer within 14 days.

Sample preparation guidelines

- Refer to the *ELISA Technical Guide* at **thermofisher.com** for detailed sample preparation procedures.
- 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

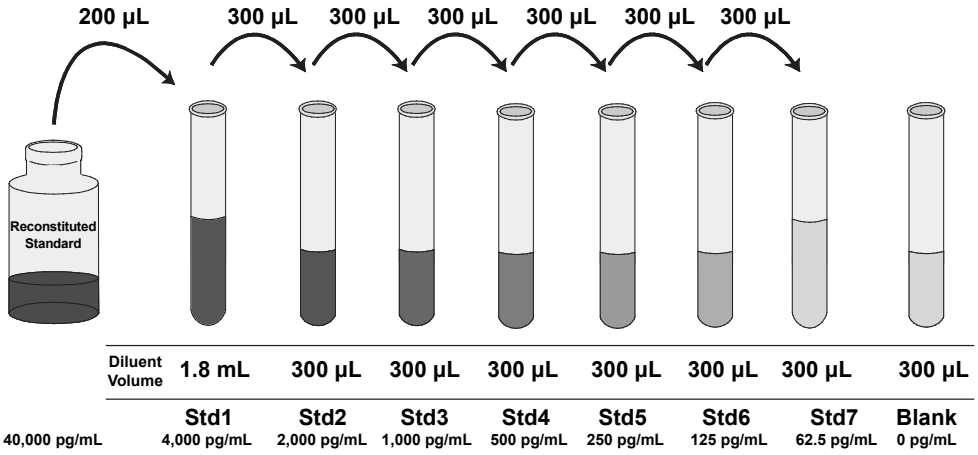
Because conditions may vary, we recommend that each investigator determine the optimal dilution for each application.

- Dilute **serum and plasma** sample 1:5 in Standard Diluent Buffer.
- Assay **cell culture supernatant** samples neat.
- Perform sample dilutions with Standard Diluent Buffer (serum/plasma or with the corresponding cell culture medium (cell culture supernatant).

Dilute standards

Note: Use glass or plastic tubes for diluting standards.

1. Reconstitute Rt Leptin Standard to 40,000 pg/mL with Standard Dilution 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 40,000 pg/mL rat leptin. **Use the standard within 15 minutes of reconstitution**.
2. Add 200 µL Reconstituted Standard to one tube containing 1.8 mL Standard Diluent Buffer and mix. Label as 4,000 pg/mL rat leptin.
3. Add 300 µL Standard Diluent Buffer to each of 7 tubes labeled as follows: 2,000, 1,000, 500, 250, 125, 62.5, and 0 pg/mL rat 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 1X Streptavidin-HRP solution

Note: Prepare 1X Streptavidin-HRP within 15 minutes of usage.

1. For each 8-well strip used in the assay, pipet 10 µL Streptavidin-HRP (100X) solution and dispense the solution into a tube containing 1 mL of Streptavidin-HRP Diluent. Mix thoroughly.
2. Return the unused Streptavidin-HRP (100X) solution to the refrigerator.

Perform ELISA (Total assay time: 4 hours)

IMPORTANT! Perform a standard curve with each assay.

- Allow all components to reach room temperature before use. Mix all liquid reagents prior to use.
- 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°C to 8°C for future use.

1 Bind antigen

a. Add 100 µL of standards, controls, or samples (see "Pre-dilute samples" on page 2) to the appropriate wells. Leave the wells for chromogen blanks empty.
b. Cover the plate with a plate cover and incubate for 2 hours at room temperature.
c. Thoroughly aspirate the solution and wash wells 4 times with 1X Wash Buffer.

2 Add Biotin Conjugate

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

3 Add Streptavidin-HRP

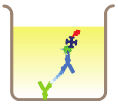
a. Add 100 µL 1X Streptavidin-HRP solution (see page 2) into each well except the chromogen blanks.
b. Cover the plate with a plate cover and incubate for 30 minutes at room temperature.
c. Thoroughly aspirate the solution from the wells and wash wells 4 times with 1X Wash Buffer.

4 Add Stabilized Chromogen

a. Add 100 µL Stabilized Chromogen to each well. The substrate solution begins to turn blue.
b. Incubate for 30 minutes at room temperature in the dark.

Note: TMB should not touch aluminum foil or other metals.

5 Add Stop Solution



Add 100 µL Stop Solution to each well. Tap the side of the plate 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 4 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 the upper limit of the standard curve in Standard Diluent Buffer (serum/plasma) or with the corresponding cell culture medium (cell culture supernatant) 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 to 4,000 pg/mL rat leptin.

Standard Rat Leptin (pg/mL)	Optical Density (450 nm)
4,000	2.87
2,000	2.23
1,000	1.55
500	0.96
250	0.53
125	0.38
62.5	0.25
0	0.12

Sensitivity

The analytical sensitivity of the assay is <20 pg/mL rat leptin. This was determined by adding two standard deviations to the mean O.D. obtained from 30 assays of the zero standard.

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)	201.8	1,010.4	1,771.8
Standard Deviation	13.9	81.1	114.3
% Coefficient of Variation	6.9	8.0	6.4

Intra-assay precision

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

Parameters	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	208.70	982.80	1797.10
Standard Deviation	11.90	50.20	101.40
% Coefficient of Variation	5.70	5.10	5.60

Expected values

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

Sample	Range (pg/mL)	Average (pg/mL)
Serum (n=29)	157–2,157	1,108
EDTA plasma (n=4)	423–1,373	847
Citrate plasma (n=4)	361–1,205	693
Heparin plasma (n=3)	2,398–2,797	2,629
Early stage pregnant serum (n=3)	1,478–2,175	1,902
Mid stage pregnant serum (n=3)	2,178–3,746	2,853
Late stage pregnant serum (n=3)	2,335–4,217	3,269

Linearity of dilution

Rat serum, EDTA plasma, citrate plasma, heparin plasma, and cell culture medium spiked with recombinant rat 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	0.99
EDTA plasma	1.00
Citrate plasma	0.97
Heparin plasma	0.99
Tissue culture media	1.00

Recovery

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

Sample	Avg % Recovery
Serum	93
EDTA plasma [1]	124
Citrate plasma[1]	111
Heparin plasma[1]	116
DMEM + 10% calf serum	108
RPMI+10% fetal bovine serum	114

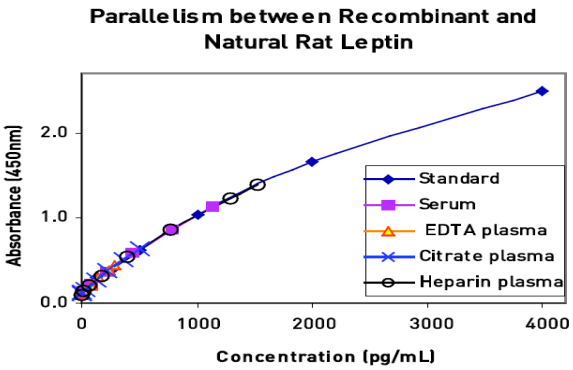
[1] Serum and plasma were prediluted 5-fold as described in **Pre-Dilute samples**.

Specificity

Buffered solutions of a panel of substances ranging in concentration from 2,000 to 50,000 pg/mL were assayed with the Rat Leptin ELISA Kit and found to have no cross-reactivity: **human** Aβ42, APO-1/FAS, Bcl-2, eotaxin, GM-CSF, GRO-α, IFN-α, IFN-γ, IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IL-13, IL-15, IL-17, IP-10, leptin, MCP-1, MCP-2, MCP-3, MIG, MIP-1α, MIP-1β, RANTES, sICAM-1, TNF-α, TRAIL, VEGF; **mouse** eotaxin, FGFb, GM-CSF, IFN-γ, IL-1α, IL-1β, IL-2, IL-3, 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; **rat** GM-CSF, IFN-γ, IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-6, IL-10, IL-12, IL-13, TNF-α, MCP-1, MIP-2, RANTES; **swine** IFN-γ, IL-1β; **bovine** leptin.

Parallelism

Random rat serum and plasma samples were serially diluted in Standard Diluent Buffer. The optical density of each dilution was plotted against the rat leptin Standard Curve. Parallelism demonstrates that the standard accurately reflects the rat leptin content in natural samples.



Cross-reactivity

Cross-reactivity with recombinant mouse leptin was determined to be 15%. Random, normal serum samples were also evaluated with the kit. No cross-reactivity was observed with goat, rabbit, monkey, or bovine serum samples. There was low cross-reactivity with human, hamster and swine, and significant cross-reactivity with mouse serum samples.

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

Bender MedSystems GmbH | Campus Vienna Biocenter 2 | 1030 Vienna, Austria
For descriptions of symbols on product labels or product documents, go to **thermofisher.com/symbols-definition**.

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