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# Product Information

**Mouse Leptin ELISA Kit**
for serum, plasma and cell culture supernatant

Catalog Number **RAB0334**
Storage Temperature –20 °C

## TECHNICAL BULLETIN

### Product Description

The Mouse Leptin ELISA (Enzyme-Linked Immunosorbent Assay) kit is an in vitro enzyme-linked immunosorbent assay for the quantitative measurement of mouse Leptin in serum, plasma and cell culture supernatants. This assay employs an antibody specific for mouse Leptin coated on a 96-well plate. Standards and samples are pipetted into the wells and Leptin present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti-mouse Leptin antibody is added. After washing away unbound biotinylated antibody, HRP conjugated streptavidin is pipetted to the wells. The wells are again washed, a TMB substrate solution is added to the wells and color develops in proportion to the amount of Leptin bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm

### Components

1. Mouse Leptin Antibody-coated ELISA Plate (Item A) - RAB0334A-EA: 96 wells (12 strips x 8 wells) coated with anti-mouse Leptin.
2. 20x Wash Buffer (Item B) - RABWASH4: 25 mL of 20x concentrated solution.
3. Lyophilized Mouse Leptin Protein Standard (Item C) - RAB0334C-1VL: 2 vials of Mouse Leptin. 1 vial is enough to run each standard in duplicate
4. Biotinylated Mouse Leptin Detection Antibody (Item F) - RAB0334D-1VL: 2 vials of biotinylated anti-Mouse Leptin. Each vial is enough to assay half the microplate.
5. HRP-Streptavidin (Item G) - RABHRP5: 200 µL 120X concentrated HRP-conjugated streptavidin.
6. ELISA Colorimetric TMB Reagent (HRP Substrate, Item H) - RABTMB3: 12 mL of 3,3,5,5'-tetramethylbenzidine (TMB) in buffer solution.
7. ELISA Stop Solution (Item I) - RABSTOP3: 8 mL of 0.2 M sulfuric acid.
8. ELISA 1x Assay/Sample Diluent Buffer A (Item D1) - RABELADA-30ML: 30 mL of diluent buffer, 0.09% sodium azide as preservative.
9. ELISA 5x Assay/Sample Diluent Buffer B (Item E1) - RABELADB-15ML: 15 mL of 5X concentrated buffer.

### Reagents and Equipment Required but Not Provided.

1. Microplate reader capable of measuring absorbance at 450 nm.
2. Precision pipettes to deliver 2 µL to 1 mL volumes.
3. Adjustable 1-25 mL pipettes for reagent preparation.
4. 100 mL and 1liter graduated cylinders.
5. Absorbent Paper
6. Distilled or deionized water.
7. Tubes to prepare sample dilutions.
8. Log-log graph paper or computer and software for ELISA data analysis
9. Tubes to prepare the positive control or sample dilutions.

### Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Material Safety Data Sheet

for information regarding hazards and safe handling practices.

**Preparation Instructions**

Reagent Preparation

1. Bring all reagents and samples to room temperature (18 – 25 ºC) before use.

2. Assay Diluent B (Item E) should be diluted 5-fold with deionized or distilled water before use.

3. Sample dilution: Assay Diluent A (Item D) should be used for dilution of serum and plasma samples. 1X Assay Diluent B (Item E) should be used for dilution of cell culture supernatant samples. The suggested dilution for normal serum/plasma is 10 - 40 fold.
   Note: Levels of Leptin may vary between different samples. Optimal dilution factors for each sample must be determined by the investigator

4. Preparation of standard: Briefly spin a vial of Item C. Add 400 µL Assay Diluent A (for serum/plasma samples) or 1X Assay Diluent B (for cell culture medium) into Item C vial to prepare a 30 ng/mL standard. Dissolve the powder thoroughly by a gentle mix. Add 20 µL Leptin standard from the vial of Item C, into a tube with 580 µL Assay Diluent A or 1X Assay Diluent B to prepare 1,000 pg/mL standard solution. Pipette 300 µL Assay Diluent A or 1X Assay Diluent B into each tube. Use the standard solution to produce a dilution series (shown below). Mix each tube thoroughly before the next transfer. Assay Diluent A or 1X Assay Diluent B serves as the zero standard (0 pg/mL)

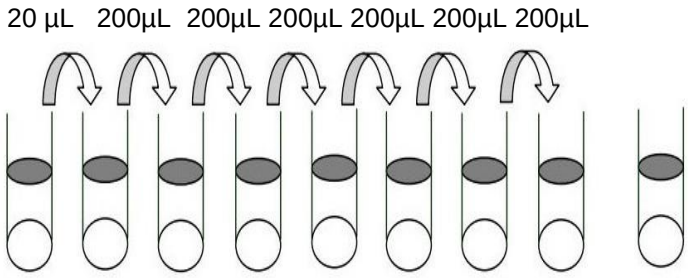
5. If the Wash Concentrate (20X) (Item B) contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute 20 mL of Wash Buffer Concentrate into deionized or distilled water to yield 400 mL of 1X Wash Buffer.

6. Briefly spin the Detection Antibody vial (Item F) before use. Add 100 µL of 1X Assay Diluent B (Item E) into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently (the concentrate can be stored at 4ºC for 5 days). The detection antibody concentrate should be diluted 120-fold with 1X Assay Diluent B (Item E) and used in step 5 of Part VI Assay Procedure.

7. Briefly spin the HRP-Streptavidin concentrate vial (Item G) and pipette up and down to mix gently before use, as precipitates may form during storage. HRP-Streptavidin concentrate should be diluted 120-fold with 1X Assay Diluent B (Item E).

   For example: Briefly spin the vial (Item G) and pipette up and down to mix gently. Add 100 µL of HRP-Streptavidin concentrate into a tube with 12 mL 1X Assay Diluent B to prepare 120 fold diluted HRP- Streptavidin solution. Mix well

**Figure 1.**




| | | Std 1 | Std 2 | Std 3 | Std 4 | Std 5 | Std 6 | Std 7 | 0 Std |
|---|---|---|---|---|---|---|---|---|---|
| Diluent volume | Item C + 400 µL | 580 µL | 300 µL | 300 µL | 300 µL | 300 µL | 300 µL | 300 µL | 300 µL |
| **Conc.** | 30 ng/mL | 1000 pg/mL | 400 pg/mL | 160 Pg/mL | 64 pg/mL | 25.6 pg/mL | 10.24 pg/mL | 4.1 pg/mL | 0 Pg/mL |

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# Product Information

**Storage/Stability**
The entire kit may be stored at -20 °C for up to 1 year from the date of shipment. Avoid repeated freeze-thaw cycles. The kit may be stored at 4 °C for up to 6 months. For extended storage, it is recommended to store at -80 °C. For prepared reagent storage, see table below.

**Procedure**

1. Bring all reagents and samples to room temperature (18 – 25 ºC) before use. It is recommended that all standards and samples be run at least in duplicate.

2. Label removable 8-well strips as appropriate for your experiment.

3. Add 100 µL of each standard (see Reagent Preparation step 3) and sample into appropriate wells. Cover wells and incubate for 2.5 hours at room temperature with gentle shaking.

4. Discard the solution and wash 4 times with 1X Wash Solution. Wash by filling each well with Wash Buffer (300 µL) using a multi-channel Pipette 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.

5. Add 100 µL of 1X prepared biotinylated antibody (Reagent Preparation step 6) to each well. Incubate for 1 hour at room temperature with gentle shaking.

6. Discard the solution. Repeat the wash as in step 4.

7. Add 100 µL of prepared Streptavidin solution (see Reagent Preparation step 7) to each well. Incubate for 45 minutes at room temperature with gentle shaking.

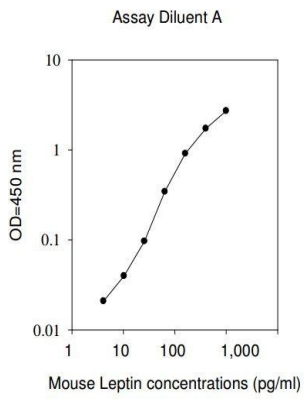
8. Discard the solution. Repeat the wash as in step 4

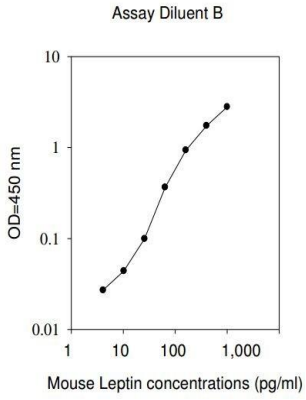
9. Add 100 µL of TMB One-Step Substrate Reagent (Item H) to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking.

10. Add 50 µL of Stop Solution (Item I) to each well. Read at 450 nm immediately

**Results**

**Calculations**
Calculate the mean absorbance for each set of duplicate standards, controls and samples,and subtract the average zero standard optical density. Plot the standard curve on log-log graph paper or using Sigma plot software, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit straight line through the standard points.




**A. Typical Data**

These standard curves are for demonstration only. A standard curve must be run with each assay.

**B. SENSITIVITY**

The minimum detectable dose of Mouse Leptin was determined to be 4 pg/mL.

Minimum detectable dose is defined as the analyte concentration resulting in an absorbance that is 2 standard deviations higher than that of the blank (diluent buffer).

**C. SPIKING AND RECOVERY**

Recovery was determined by spiking various levels of Mouse Leptin into the sample types listed below. Mean recoveries are as follows

| Sample Type | Average % Recovery | Range (%) |
|---|---|---|
| Serum | 93.29 | 82-102 |
| Plasma | 95.38 | 84-104 |
| Cell culture media | 94.47 | 83-103 |

**D.LINEARITY**

| Sample Type | | Serum | Plasma | Cell Culture Media |
|---|---|---|---|---|
| 1:2 | Average % of Expected | 93 | 94 | 95 |
| | Range (%) | 83-103 | 83-103 | 84-103 |
| 1:4 | Average % of Expected | 95 | 93 | 96 |
| | Range (%) | 84-104 | 82-102 | 83-103 |

**E. REPRODUCIBILITY**

Intra-Assay CV%: <10%
Inter-Assay CV%: <12%

**SPECIFICITY**

This ELISA kit shows no cross-reactivity with any of the cytokines tested: Mouse CD30, L CD30, T CD40, CRG-2, CTACK, CXCL16, Eotaxin , Eotaxin-2, Fas Ligand, Fractalkine, GCSF, GM-CFS, IGFBP-3, IGFBP-5, IGFBP-6, IL-1 alpha, IL-1 beta, IL-2, IL-3, IL-3 Rb, IL-4, IL-5, IL-6, IL-9, IL-10, IL-12 p40/p70, IL-12 p70, IL-13, IL-17, KC, Leptin R, LIX, L-Selectin, Lymphotactin, MCP-1, MCP-5, M-CSF, MIG, MIP-1 alpha, MIP-1 gamma, MIP-2, MIP-3 beta, MIP-3 alpha, PF-4, P-Selectin, RANTES, SCF, SDF- 1alpha, TARC, TCA-3, TECK, TIMP-1, TNF-alpha, TNF RI, TNF RII, TPO, VCAM-1, VEGF

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**Appendix**

Troubleshooting Guide

| Problem | Cause | Solution |
|---|---|---|
| Poor standard curve | Inaccurate pipetting | Check pipettes |
| | Improper standard dilution | Ensure a brief spin of Item C and dissolve the powder thoroughly with gentle mixing. |
| Low signal | Too brief incubation times | Ensure sufficient incubation time; Procedure, step 3 may be done at overnight at 4 °C with gentle shaking (note: may increase overall signals including background). |
| | Inadequate reagent volumes or improper dilution | Check pipettes and ensure correct preparation |
| Large CV | Inaccurate Pipetting<br>Air Bubbles in well | Check Pipettes<br>Remove bubbles in well |
| High background | Plate is insufficiently washed | Review the manual for proper wash. If using a plate washer, check that all ports are unobstructed. |
| | Contaminated wash buffer | Make fresh wash buffer |
| Low sensitivity | Improper storage of the ELISA kit | Store the standard at <–70 °C after reconstitution, others at 4 °C. Keep substrate solution protected from light |
| | Stop solution | Stop solution should be added to each well before reading plate |

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