

Product Information

Leptin (human) ELISA Kit

I. Kit Contents:

Components	K2191-96 96 assays
Standard diluent	6 ml
20X Wash buffer	25 ml
Chromogen Solution A	6 ml
Chromogen Solution B	6 ml
HRP-Conjugate Reagent	10 ml
Micro ELISA strip-plate	1 unit
Plate sealers	2 units
Sample diluent	6 ml
Standard (2700 pg/ml)	0.5 ml
Stop Solution	6 ml

II. Introduction:

The Human Leptin ELISA kit is an enzyme-linked immunosorbent assay for quantitative detection of human Leptin in vitro. This assay utilizes an human Leptin specific antibody coated on a 96-well plate. Standards and samples are pipetted into wells. The Leptin antibody in the sample is attached to the wells by the immobilized antibody. The wells are washed and biotinylated anti-human Leptin antibody is added. HRP-conjugated streptavidin is pipetted to the wells following washing away of the unbound biotinylated antibody. 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. This ELISA kit shows no cross-reactivity with any of the cytokines tested. The detection limit of the Leptin is normally less than 2 pg/ml. Detection Range: 2 pg/ml - 400 pg/ml.

III. Application:

Quantitative protein detection, establishing normal range, validation of antibody array results.

IV. Specificity:

The antibody pair provided in this kit recognizes human Leptin.

V. Sample Type:

Serum & plasma.

Cell culture supernatants.

Urine.

VI. User Supplied Reagents and Equipment:

Microplate reader capable of measuring absorbance at 450 nm.

Absorbent paper.

Distilled or deionized water.

VII. Storage and Handling:

May be stored for up to 6 months at 2°C to 8°C from the date of shipment. Standard (recombinant protein) should be stored at -20°C or -80°C (recommended at -80°C) after reconstitution. Opened Microplate Wells or reagents may be stored for up to 1 month at 2°C to 8°C. Return unused wells to the pouch containing desiccant pack, reseal along entire edge. Note: the kit can be used within one year if the whole kit is stored at -20°C. Avoid repeated freeze-thaw cycles.

VIII. Reagent Preparation:

Bring all reagents and samples to room temperature (18 - 25°C) before use.

Sample dilution: If your samples need to be diluted, Assay Diluent A (Item D) should be used for dilution of serum/plasma samples. 1x Assay Diluent B (Item E) should be used for dilution of culture supernatants and urine. Suggested dilution for normal serum/plasma: 20 - 50 fold.

Please note that levels of the target protein may vary between different specimens. Optimal dilution factors for each sample must be determined by the investigator.

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

Preparation of standard: Briefly spin the vial of Item C then add 440 µl Assay Diluent A (for serum/plasma samples) or 1X Assay Diluent B (for cell culture supernates/urine) into Item C vial to prepare a 220 ng/ml standard. Dissolve the powder thoroughly by a gentle mix. Add 2 µl Leptin standard from the vial of Item C, into a tube with 1098 µl Assay Diluent A or 1X Assay diluent B to prepare a 400 pg/ml standard solution. Pipette 300 µl Assay Diluent A or 1X Assay diluent B into each tube. Use the stock standard solution to produce a dilution series (shown). Mix each tube thoroughly before the next transfer. Assay Diluent A or 1X Assay Diluent B serves as the zero standard (0 pg/ml).

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.

Briefly spin the Detection Antibody vial (Item F) before use. Add 100 µl of 1x Assay Diluent B into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently (the concentrate can be stored at 4°C for 5 days). The detection antibody concentrate should be diluted 80-fold with 1x Assay Diluent B and used in step 4 of Assay Protocol.

Briefly spin the HRP-Streptavidin concentrate vial (Item G) and pipette up and down to mix gently before use. HRP-Streptavidin concentrate should be diluted 160-fold with 1x Assay Diluent B.

IX. Assay Protocol:

1. Bring all reagents and samples to room temperature (18 - 25°C) before use. It is recommended that all standards and samples be run at least in duplicate.
2. Add 100 µl of each standard and sample into appropriate wells. Cover well and incubate for 2.5 hours at room temperature or overnight at 4°C with gentle shaking.
3. 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.
4. Add 100 µl of 1x prepared biotinylated antibody (see Reagent Preparation) to each well. Incubate for 1 hour at room temperature with gentle shaking. Discard the solution. Repeat the wash as in step 3.
5. Add 100 µl of prepared Streptavidin solution (see Reagent Preparation) to each well. Incubate for 45 minutes at room temperature with gentle shaking. Discard the solution. Repeat the wash as in step 3.
6. 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.
7. Add 50 µl of Stop Solution (Item I) to each well. Read at 450 nm immediately.

8. Calculation: 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.

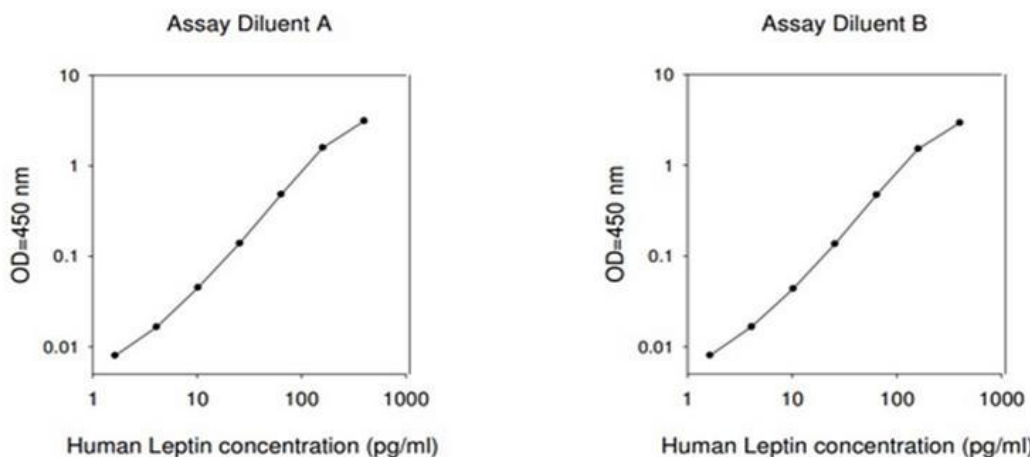


Figure 1: Standard Curve: These standard curves are for demonstration only. A standard curve must be run with each assay.

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