NOVATEINBIO

Accelerates Research and Development

Human Leptin ELISA Kit

Catalog number: NR-R10001 (96 wells)

The kit is designed to detect the level of Human Leptin in cell culture supernatant, serum, plasma and other suitable sample solution

FOR **RESEARCH USE ONLY**. NOT FOR DIAGNOSTIC OR THERAPEUTIC PURPOSES

Background

Leptin is a secreted hormone made by fat cells, which regulates the amount of fat stored in the body. It may function as part of a signaling pathway that acts to regulate the size of the body fat depot. An increase in the level of LEP may act directly or indirectly on the CNS to inhibit food intake and/or regulate energy expenditure as part of a homeostatic mechanism to maintain constancy of the adipose mass. Leptin does this by adjusting both the sensation of hunger, and adjusting energy expenditures. Although regulation of fat stores is deemed to be the primary function of leptin, it also plays a role in other physiological processes, as evidenced by its multiple sites of synthesis other than fat cells, and the multiple cell types beside hypothalamic cells which have leptin receptors.

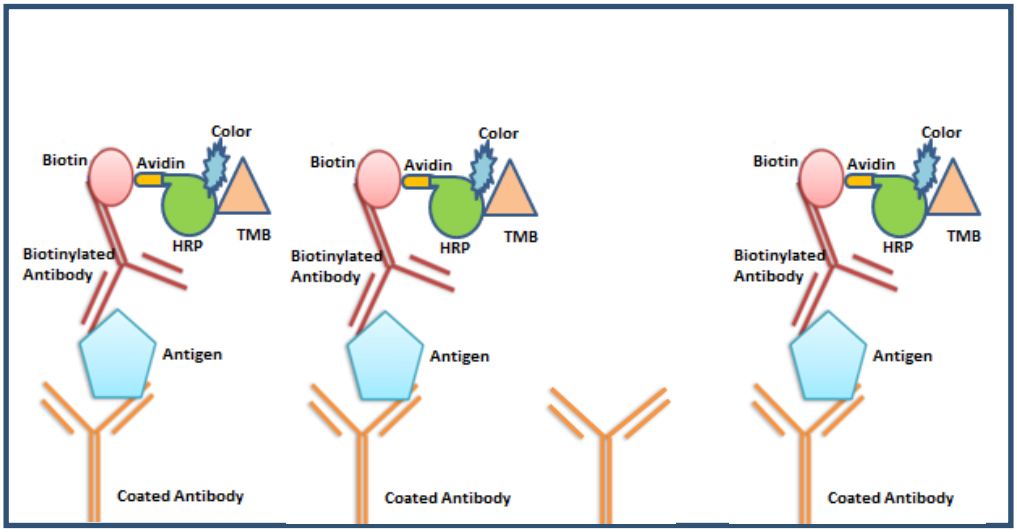
Intended use

The kit is used to quantify the Human Leptin in cell culture supernatant, serum, plasma and other suitable sample solution.

Standard range	0 - 100 ng/ml
Sensitivity	0.3 ng /ml
Assay time	3.5 hours
Validity	12 months
Store at	2-8 °C

Assay principle

This human Leptin ELISA Kit was based on standard sandwich enzyme-linked immunosorbent assay technology. Human leptin specific antibodies were precoated onto 96-well plates. The leptin specific detection antibodiy was biotinylated. The test samples and biotinylated detection antibodies were added to the wells subsequently and then followed by washing the plate. Streptavidin-HRP was added and unbound conjugates were washed away with wash buffer. HRP substrate TMB was used to visualize HRP enzymatic reaction. TMB was catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. The density of yellow is proportional to the leptin amount of sample captured in plate.



Materials supplied

1	Microelisa Stripplate	96 well
2	Human leptin Standard set	6 vials
3	Biotinylated leptin Detection antibody	1 vial (11ml)
4	20 X Wash Solution	25 ml
5	Assay diluent	12 ml
6	Strepavidin-HRP	1 vial (11ml)
7	TMB substrate	12 ml
8	Stop Solution	12 ml
9	Plate sealer	2
10	Package insert	1

Note: Standard (S1 - S6) concentration was followed by: 0, 2, 5, 25, 50 and 100 ng/ml when reconstituted with 0.5ml distilled water.

Materials required but not supplied

- Standard plate reader capable of measuring absorbance at 450 nm.
- Adjustable pipettes and disposable pipette tips.
- Distilled water.
- Multi-channel pipettes, manifold dispenser or automated microplate washer.
- Absorbent paper.
- Materials used for sample preparation.

Sample collection and storage

- **Serum** - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before a centrifugation for 15minutes at approximately 1000 x g. Remove serum and perform the assay immediately or aliquot and store samples at -20 °C or -80°C.
- **Plasma** - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1000 x g at 2-8°C within 30minutes of collection. Store samples at -20°C or -80°C.Avoid repeated freeze-thaw cycles.
- **Cell culture fluid and other biological fluids** - Remove particulates by centrifugation and assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.

  NOTE: Serum, plasma, and cell culture fluid samples to be used within 7 days may be stored at 2-8°C, otherwise samples must be stored at -20°C(≤2months) or -80°C (≤6months) to avoid loss of bioactivity and contamination. Avoid freeze-thaw cycles. When performing the assay, warm up samples to room temperature slowly. **DO NOT USE HEAT-TREATED SAMPLES**.

Sample Preparation

- Novateinbio is only responsible for the kit itself, but not for the samples consumed during the assay. The user should calculate the possible amount of the samples used in the whole test. Please reserve sufficient amount of samples in advance.

- Please predict the concentration before assaying. If values for these are not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.
- If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.
- Owing to the possibility of mismatching between antigen from other resource and antibody used in our kits (e.g., antibody targets conformational epitope rather than linear epitope), so me native or recombinant proteins from other manufacturers may not be recognized by our products.
- Fresh samples without long time storage is recommended for the test. Otherwise, protein degradation and denaturalization may occur in those samples and finally lead to inaccurate results.

Reagent Preparation

- Bring all kit components and samples to room temperature (18-25 °C) before use.
- **Standard**: Reconsitute each vial with 0.5ml of distilled water. All reconstituted standards are stable at 2-8°C for up to 4 weeks.
- **Wash Solution** -Dilute 25 mL of Wash Solution concentrate (20 ×) with 475 mL of deionized or distilled water to prepare 500 mL of Wash Solution (1 ×).

Assay procedures

1. Prepare all the Standards before starting assay procedure (Please read Reagents Preparation). It is recommended that all Standards and Samples should be added in duplicate to the Microtiter Plate.
2. Secure the desired numbers of coated wells in the holder.
3. Take the Standards and agitate gently prior to use then add 15µl of Standards or Samples to the appropriate well of the antibody pre-coated Microtiter Plate.
4. Add 100µl of assay buffer to each well. Mix well. **Mixing well in this step is important.** Cover and incubate the plate for 2 hours at room temperature.
5. Wash the Microtiter Plate using one of the specified methods indicated below:

   Manual Washing: Remove incubation mixture by aspirating contents of the plate into a sink or proper waste container. Fill in each well completely with diluted wash solution, and then aspirate contents of the plate into a sink or proper waste container. Repeat this procedure five times for a total of FIVE washes. After washing, invert plate, and blot dry by hitting the plate onto absorbent paper or paper towels until no moisture appears.

Note: Hold the sides of the plate frame firmly when washing the plate to assure that all strips remain securely in frame. Complete removal of liquid at each step is essential to good performance.

Automated Washing: Wash plate FIVE times with diluted wash solution (350-400µl/well/wash) using an auto washer. After washing, dry the plate as above. It is recommended that the washer be set for a soaking time of 10 seconds and shaking time of 5 seconds between each wash.

6. Add 100 µl of Biotinylated leptin Detection antibody solution into each well and incubate for 30 minutes at room temperature.
7. Wash the plate as described in step 5.
8. Add 100 µl of Strepavidin-HRP solution into each well and incubate for 30 minutes at room temperature.
9. Wash the plate as described in step 5.
10. Add 100µl of TMB Substrate solution into each well. Cover and incubate for 10 - 30 minutes at room temperature. (Protect from light. Do not over develop).
11. Add 50µl Stop Solution to each well. Mix well.
12. Read the Optical Density (O.D.) at 450 nm using a microtiter plate reader immediately.

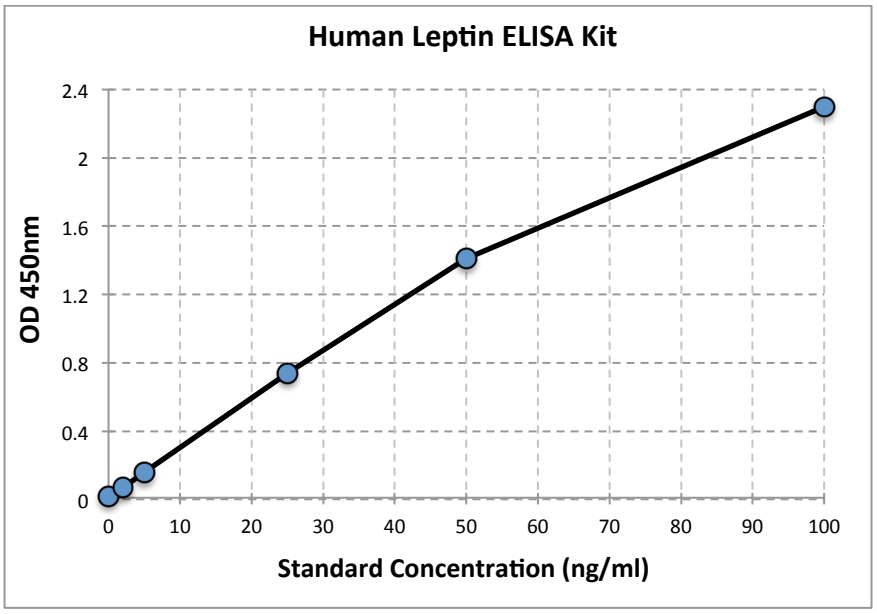
Important notes

- The operation should be carried out in strict accordance with the provided instructions.
- Store the unused strips in a sealed foil bag at 2-8°C.
- Always avoid foaming when mixing or reconstituting protein solutions.
- Pipette reagents and samples into the center of each well, avoid from bubbles.
- The samples should be transferred into the assay wells within 15 minutes of dilution.
- We recommended that all standard, testing samples are tested in duplicate.
- Use serial diluted sample is recommended for first test to get the best dilution factor.
- If the blue color develops too shallow after 15 minutes incubation with the substrates, it may be appropriate to extend the incubation time. (Do not over develop)
- Avoid cross-contamination by changing tips, using separate reservoirs for each reagent.
- Avoid using the suction head without extensive wash.
- Do not mix the reagents from different batches.
- Stop Solution should be added in the same order of the Substrate solution.
- TMB substrate solution is light sensitive. Avoid prolonged exposure to the light.

Result calculation

- The standard curve is used to determine the amount of samples.
- First, calculate the mean O.D. value for each standard and sample. All O.D. values, are subtracted by the mean value of the blank control before result interpretation.
- Construct a standard curve by plotting the average O.D. for each standard on the vertical (Y) axis against the concentration on the horizontal (X) axis, and draw a best fit curve e using graph paper or statistical software to generate a four paramater logistic (4-PL) curve-fit or logit-log linear regression curve. The data may be linearized by plotting the log of the concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis.
- Any variation in operator, pipetting and washing technique, incubation time or temperature, and kit age can cause variation in result. **A standard curve should be generated for each assay.**

Typical data:



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