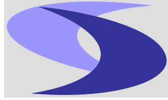

Signosis
BioSignal Capture

Mouse Leptin ELISA

Catalog Number EA-2202 **(For Research Use Only)**

Introduction

Mouse leptin plays a key role in regulating energy intake and energy expenditure, including the regulation (decrease) of appetite and (increase) of metabolism. Leptin is produced by adipose tissue and the level of circulating leptin is directly proportional to the total amount of fat in the body. Once leptin has bound to the Ob-Rb receptor, it activates Stat3, which is phosphorylated and travels to the nucleus where it mediates gene expression. One of the main effects on gene expression is the down-regulation of the expression of endocannabinoids, responsible—among their many other functions—for increasing appetite. There are other intracellular pathways activated by leptin, but less is known about how they function in this system. Although leptin is a circulating signal that reduces appetite, in general, obese people who are resistant to the effects of leptin have an unusually high circulating concentration of leptin, in the same way that people with type 2 diabetes are resistant to the effects of insulin (1). Leptin is also found to stimulate endothelial cell proliferation and angiogenesis.

Principle of the assay

Leptin ELISA is based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay utilizes rabbit anti-mouse leptin antibodies for immobilization on the microtiter wells and biotinated rabbit anti-mouse leptin antibodies along with streptavidin conjugated to horseradish peroxidase (HRP) for detection. The test sample is allowed to react simultaneously with the two antibodies, resulting in the leptin molecules being sandwiched between the solid phase and enzyme-linked antibodies. After incubation, the wells are washed to remove unbound-labeled antibodies. A HRP substrate, TMB, is added to result in the development of a blue color. The color development is then stopped with the addition of Stop Solution changing the color to yellow. The concentration of leptin is directly proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm.

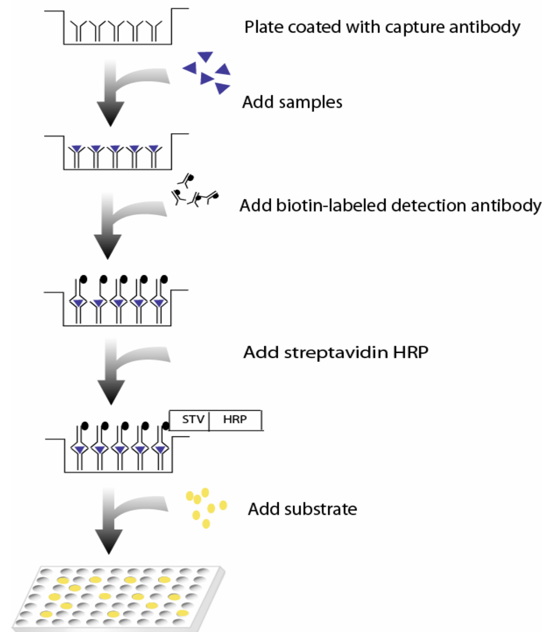

Diagram of ELISA

Materials provided with the kit

- 96 well microplate coated with rabbit anti-mouse leptin antibody (4°C).
- Biotin labeled rabbit anti-mouse leptin antibody (-20°C).
- Streptavidin-HRP conjugate (4°C).
- Recombinant mouse leptin standard (1000ng/ml) (-20°C).
- 1X Diluent buffer (4°C).
- 5X Assay wash buffer (RT)
- Substrate (4°C).
- Stop Solution (4°C).

Material required but not provided

- Microplate reader capable of measuring absorbance at 450 nm
- Deionized or distilled water.

Reagent preparation before starting experiment

- Dilute the 5x Assay wash buffer to 1x buffer
  40ml 5x Assay wash buffer
  160ml ddH2O
- Dilute 500 times of mouse recombinant leptin (1000ng/ml) with 1X Diluent buffer to 2000pg/ml and then 2-fold serial dilutions.
- Dilute 400 times of biotin labeled rabbit anti-mouse leptin antibodies with 1X Diluent buffer before use.
- Dilute 200 times of streptavidin-HRP with 1X Diluent buffer before use.

Assay procedure

1. Cut the sealing film over the plate and remove it from the desired number of wells. Make sure the rest of wells are well sealed.
2. Add 100 µl of Standard, control, or sample per well and incubate for 1 hour at room temperature with gentle shaking.
3. Aspirate each well and wash by adding 200µl of 1X Assay wash buffer. Repeat the process three times for a total of three washes. Complete removal of liquid at each wash. After the last wash, remove any remaining liquid by inverting the plate against clean paper towels.
4. Add 100 µl of diluted biotin-labeled rabbit anti-mouse leptin antibodies to each well and incubate for 1 hour at room temperature with gentle shaking.
5. Repeat the aspiration/wash as in step 3.
6. Add 100 µl of diluted streptavidin-HRP conjugate to each well and incubate for 45 min at room temperature with gentle shaking.
7. Repeat the aspiration/wash as in step 3.
8. Add 100µl of substrate to each well and incubate for 5-30 minutes.
9. Add 50µl of Stop solution to each well. The color in the wells should change from blue to yellow.
10. Determine the optical density of each well with a microplate reader at 450 nm within 30 minutes.

References

1. Considine RV, Sinha MK, Heiman ML, Kriauciunas A, Stephens TW, Nyce MR, Ohannesian JP, Marco CC, McKee LJ & Bauer TL (1996). "Serum Immunoreactive-Leptin Concentrations in Normal-Weight and Obese Humans". *N Engl J Med* **334** (5): 292-295. PMID 8532024.

Example of standard curve