Signosis
Innovative Plate Assay Solutions

Rat Leptin ELISA

Catalog Number EA-3013 **(For Research Use Only)**

Introduction

Rat 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-rat leptin antibodies for immobilization on the microtiter wells and biotinated rabbit anti-rat 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.

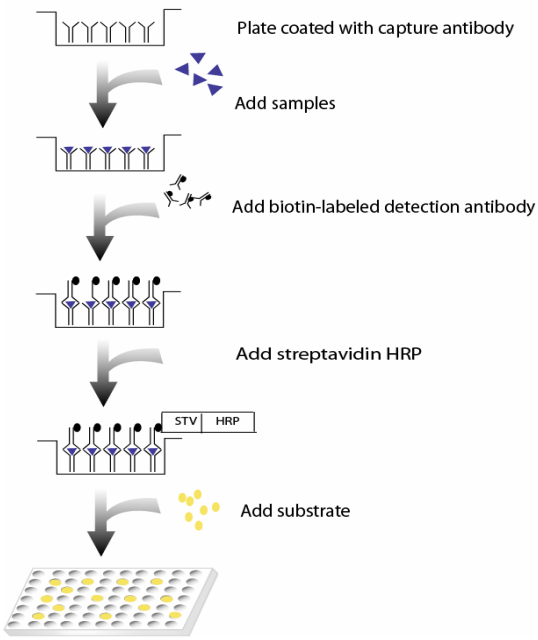


Diagram of ELISA

Materials provided with the kit

Component	Qty	Store at
96-Well 12 strip Plate coated with rabbit anti-rat Leptin antibody	1	4°C
Biotin-labeled rabbit anti-rat Leptin antibody	200µL	-20°C
Recombinant rat Leptin standard (400ng/ml)	10µL	-20°C
Streptavidin-HRP conjugate	50µL	4°C
1xDiluent buffer	40mL	4°C
5X Assay wash buffer	40mL	4°C
Substrate	10mL	4°C
Stop solution	5mL	RT

Material required but not provided

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

Reagent preparation before starting experiment

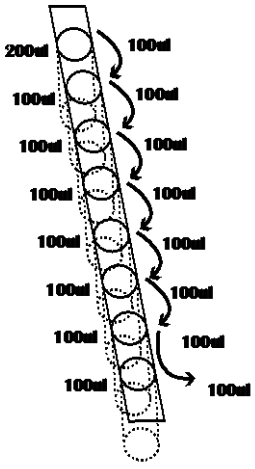
- Dilute the 5x Assay wash buffer to 1x buffer:
  - 40ml 5x Assay wash buffer
  - 160ml ddH2O
- Dilute 100 times of Rat recombinant Leptin (400ng/ml) to 4000pg/ml by adding 2µl Rat recombinant Leptin in 200µl 1x Diluent Buffer and then 2-fold serial dilutions (See Step 2 below for detailed instruction)
- Dilute 400 times of biotin labeled goat anti-rat Leptin antibodies with 1X Diluent buffer before use.
- Dilute 200 times of streptavidin-HRP with 1X Diluent buffer before use.

Sample preparation before starting experiment

- For **cell culture medium samples**, add 100µl directly to the well.
- For **cell lysate samples**, use cell lysis buffer (Catalog# EA-0001). Follow protocol in Cell Lysate Buffer User Manual.
- For **serum or plasma samples**, we recommend a 1:10 dilution with 1X diluent buffer, for example, add 80ul sample in 720ul 1X diluent buffer. When serum-containing conditional media is required, be sure to use serum as control.

Assay procedure

1. Calculate the number of samples to decide how many strips need to be used.
2. Add 100µl of Standard, control, or sample per well and incubate for 1 hour at room temperature with gentle shaking.



a. Add 200ul 1X Diluent buffer to the 1st well. Add 100ul 1X Diluent Buffer to the rest wells of strip.
b. Add appropriate amount of protein recombinant (follow instruction in "Reagent Preparation")
c. Mix dilutions in 1st well and transfer 100ul from the 1st well to the next dilution. (See picture) Incubate each well for 1 hr 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-rat 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