Rabbit Leptin (LEP) ELISA Kit

Catalog No. CSB-E06971Rb

(96T)

- This immunoassay kit allows for the in vitro quantitative determination of **rabbit LEP** concentrations in **serum, plasma**.
- **Expiration date** **six** months from the date of manufacture
- **FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.**

INTRODUCTION

Leptin is a 16 kDa protein hormone that plays a key role in regulating energy intake and energy expenditure, including appetite and metabolism. Leptin is one of the most important adipose derived hormones. The Ob(Lep) gene (Ob for obese, Lep for leptin) is located on chromosome 7 in humans. Leptin interacts with six types of receptors (Ob-Ra–Ob-Rf, or LepRa-LepRf) which in turn are encoded by a single gene, LEPR. Ob-Rb is the only receptor isoform that can signal intracellularly via the Jak-Stat and MAPK signal transduction pathways, and is present in hypothalamic nuclei.

In addition to being a biomarker for body fat, serum leptin levels also reflect individual energy balance. Several studies have shown that fasting or following a very low calorie diet (VLCD) lowers leptin levels. It might be that on short term leptin is an indicator of energy balance. This system is more sensitive to starvation than to overfeeding, i.e. leptin levels do not rise extensively after overfeeding. It might be that the dynamics of leptin due to an acute change in energy balance are related to appetite and eventually to food intake. Although this is a new hypothesis, there are already some data that support it.

Leptin binds to the ventromedial nucleus of the hypothalamus, known as the "appetite center." Leptin signals to the brain that the body has

had enough to eat, or satiety. A very small group of humans possess homozygous (same on both of the pair) mutations for the leptin gene which leads to a constant desire for food, resulting in severe obesity. This condition can be treated successfully by the administration of recombinant human leptin. Thus, circulating leptin levels give the brain input regarding energy storage so it can regulate appetite and metabolism. Leptin works by inhibiting the activity of neurons that contain neuropeptide Y (NPY) and agouti-related peptide (AgRP), and by increasing the activity of neurons expressing α-melanocyte-stimulating hormone (α-MSH). Leptin is also strongly linked with angiogenesis, increasing Vascular endothelial growth factor (VEGF) levels.

Although leptin is a circulating signal that reduces appetite, in general, obese people have an unusually high circulating concentration of leptin. These people are said to be resistant to the effects of leptin, in much the same way that people with type 2 diabetes are resistant to the effects of insulin. The high sustained concentrations of leptin from the enlarged adipose stores result in leptin desensitization. The pathway of leptin control in obese people might be flawed at some point so the body doesn't adequately receive the satiety feeling subsequently to eating.

PRINCIPLE OF THE ASSAY

The microtiter plate provided in this kit has been pre-coated with an antibody specific to LEP. Standards or samples are then added to the appropriate microtiter plate wells with a Horseradish Peroxidase (HRP)-conjugated monoclonal antibody preparation specific for LEP and incubated. Then substrate solution A and B are added to each well. Only those wells that contain LEP, HRP-conjugated antibody will exhibit a change in color. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The concentration of LEP in the samples is then determined by comparing the O.D. of the samples to the standard curve.

DETECTION RANGE

2.85 ng/ml-50 ng/ml. The standard curve concentrations used for the ELISA's were 50 ng/ml, 28.6 ng/ml, 11.4 ng/ml, 5.7 ng/ml, 2.85 ng/ml

SPECIFICITY

This assay recognizes rabbit LEP No significant cross-reactivity or interference was observed.

SENSITIVITY

The minimum detectable dose of rabbit LEP is typically less than 1.8 ng/ml.

The sensitivity of this assay, or Lower Limit of Detection (LLD) was defined as the lowest concentration that could be differentiated from zero.

MATERIALS PROVIDED

Reagent	Quantity
Assay plate	1
Standard(S_1-S_5)	5
HRP-conjugate	1 x 6 ml
Wash Buffer	1 x 15 ml (20×concentrate)
Substrate A	1 x 7 ml
Substrate B	1 x7 ml
Stop Solution	1 x 7 ml

Standard	S1	S2	S3	S4	S5
Concentration (ng/ml)	2.85	5.7	11.4	28.6	50

STORAGE

1. Unopened test kits should be stored at 2-8°C upon receipt and the microtiter plate should be kept in a sealed bag to minimize exposure to damp air. The test kit may be used throughout the expiration date of the kit. Refer to the package label for the expiration date.
2. Opened test kits will remain stable until the expiring date shown, provided it is stored as prescribed above.
3. A microtiter plate reader with a bandwidth of 10 nm or less and an optical density range of 0-3 OD or greater at 450nm wavelength is acceptable for use in absorbance measurement.

REAGENT PREPARATION

1. Bring all reagents and plate to room temperature for at least 30 minutes before use. Unused wells need store at 2-8°C and avoid sunlight.
2. **Wash Buffer** If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 15 ml of Wash Buffer Concentrate into deionized or distilled water to prepare 300 ml of Wash Buffer.

Precaution: The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.

SAMPLE COLLECTION AND STORAGE

- **Serum** Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 g. Remove serum and assay immediately or aliquot and store samples at -20° C. Avoid repeated freeze-thaw cycles.
- **Plasma** Collect plasma using citrate, EDTA, or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 g within 30 minutes of collection. Assay immediately or aliquot and store samples at -20°C. Avoid repeated freeze-thaw cycles.

Note: Grossly hemolyzed samples are not suitable for use in this assay.

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all samples, standards, and controls be assayed in duplicate.

1. Reconstitute the every **Standard** with 0.5 ml of distilled water.
2. Set a Blank well without any solution. Add 50μl of Standard or Sample per well. Standard need test in duplicate.
3. Add 50μl of **HRP-conjugate** to each well (not to Blank well). Mix well and then incubate for 2 hour at 37°C.
4. Complete remove the liquid. Then fill each well with **Wash Buffer** (about 200μl), stay for 10 seconds and Spinning. Repeat the process for a total of three washes. 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 50μl of **Substrate A** and 50μl of **Substrate B** to each well, mix well. Incubate for 15 minutes at 37°C. Keeping the plate away from drafts and other temperature fluctuations in the dark.
6. Add 50μl of **Stop Solution** to each well. If color change does not appear uniform, gently tap the plate to ensure thorough mixing.
7. Determine the optical density of each well within 10 minutes, using a microplate reader set to 450 nm.

CALCULATION OF RESULTS

Using the professional soft "Curve Exert 1.3" to make a standard curve is recommended, which can be downloaded from our web.

Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density. Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the x-axis against the concentration on the y-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the LEP concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

LIMITATIONS OF THE PROCEDURE

- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- It is important that the Calibrator Diluent selected for the standard curve be consistent with the samples being assayed.

- If samples generate values higher than the highest standard, dilute the samples with the appropriate Calibrator Diluent and repeat the assay.
- Any variation in Standard Diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the Immunoassay, the possibility of interference cannot be excluded.

TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- When using an automated plate washer, adding a 30 second soak period following the addition of wash buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.

- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.