Human Leptin ELISA Kit

Enzyme Immunoassay for the quantification of human Leptin in human serum and plasma

Catalog number: ARG80831

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INTRODUCTION

Leptin, the product of the ob gene, is a recently discovered single-chain proteohormone with a molecular weight of 16 kD, which is thought to play a key role in the regulation of body weight. Its amino acid sequence exhibits no major homologies with other proteins. Leptin is almost exclusively produced by differentiated adipocytes. It acts on the central nervous system, in particular the hypothalamus, thereby suppressing food intake and stimulating energy expenditure. Leptin receptors- alternatively spliced forms exist that differ in length - belong to the cytokine class I receptor family. They are found ubiquitously in the body indicating a general role of leptin, which is currently not fully understood. A circulating form of the leptin receptor exists which acts as one of several leptin binding proteins. Besides its metabolic effects, leptin was shown to have a strong influence on a number of endocrine axes. In male mice, it blunted the starvation-induced marked decline of LH, testosterone, thyroxine and the increase of ACTH and corticosterone. In female mice, leptin prevented the starvation-induced delay in ovulation. Ob/ob mice, which are leptindeficient due to an ob gene mutation, are infertile. This defect could be corrected by administration of leptin, but not through weight loss due to fasting, suggesting that leptin is pivotal for reproductive functions.

All these actions may, at least in part, be explained by the suppressive effect of leptin on neuropeptide Y (NPY) expression and secretion by neurons in the arcuate nucleus. NPY is a strong stimulator of appetite and is known to be involved in the regulation of various pituitary hormones, e.g. suppression of GH through stimulation of somatostatin, suppression of gonadotropins or stimulation of the pituitary-adrenal axis.

The most important variable that determines circulating leptin levels is body fat mass. Obviously, under conditions of regular eating cycles, leptin reflects the proportion of adipose tissue showing an exponential relationship. This constitutive synthesis of leptin is modulated by a number of non-hormonal and hormonal variables. Stimulators in both rodents and humans are overfeeding, insulin and glucocorticoids. Suppression has been shown for fasting, cAMP and beta-3-adrenoceptor agonists. From these findings it becomes clear that leptin is an integral component of various metabolic and endocrine feedback loops.

For clinical purposes, it is important to note that serum leptin levels show a moderate circadian variation with a peak during the night at about 2 a.m. The leptin values at this time are about 30 to 100 % higher than the levels measured in the morning or early afternoon. This variation together with the influence of food intake needs to be taken into account, when blood samples are collected. Under fairly standardized conditions, i.e. normal eating cycles and blood sampling in the morning or early afternoon, a single leptin measurement is informative.

For the appropriate interpretation of measured leptin levels, reference ranges are required. Because body fat mass is the major confounding variable, these ranges should be referred to measures of the percentage body fat such as body mass index (BMI) or percent body fat determined by, e.g., bioelectric impedance assessment (BIA). Leptin levels are higher in females than in males and an age dependence was shown in children and adolescents.

Therefore, reference ranges referring to measures of body fat should be stratified according to gender and pubertal development.

Leptin levels are high in most obese patients suggesting the presence of leptin insensitivity. In a small percentage of patients, however, leptin levels have been

found inappropriately low with respect to their fat mass. It remains for future studies to prove that these patients represent a new pathophysiologic entity: leptin deficiency. Since leptin has also been shown to be of great importance for reproductive functions, possible new pathophysiologic mechanisms may be discovered relating infertility to insufficient leptin production. The discovery of leptin has released an avalanche of research activities seeking to understand the regulation and actions of this new hormone. Most importantly, it has provided a key to better understand the physiology of body weight regulation and to unveil possible pathophysiologic mechanisms in both obesity and eating disorders. Further, it may provide new insights into certain causes of infertility.

PRINCIPLE OF THE ASSAY

This assay employs the enzyme immunoassay technique. A highly specific Leptin antibody has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any Leptin present is bound by the immobilized antibody. After washing away any unbound substances, a mixture of biotin-conjugated antibody specific for Leptin and streptavidin conjugated to Horseradish Peroxidase (HRP) is added to each well and incubate. After washing away any unbound antibody-enzyme reagent, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of Leptin bound in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450 nm ±2 nm. The concentration of Leptin in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the kit at 2-8°C after receipt until its expiry date. The lyophilized reagents should be stored at –20 °C after reconstitution. Avoid repeated thawing and freezing.

Component	Quantity	Storage information
Antibody-coated microplate	8 X 12 strips	4°C.
Standards A-E (1; 10; 25; 50; 100 ng/ml)	5 vials (Lyophilized)	4°C
Control 1 (acc. Range: 9.04-13.56 ng/ml)	1 vial (Lyophilized)	4°C
Control 2 (acc. Range: 21.04-31.57 ng/ml)	1 vial (Lyophilized)	4°C
Antibody-HRP-Conjugate (mixture of biotinylated anti-Leptin antibody and HRP-Streptavidin)	12 ml (Ready-to-use)	4°C
Diluent Buffer	25 ml (Ready-to-use)	4°C
20X Wash buffer	50 ml	4°C
TMB substrate	12 ml (Ready-to-use)	4°C
STOP solution	12 ml (Ready-to-use)	4°C
Adhesive films	2	4°C or RT

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm (optional: ≥ 590 nm as reference wave length)
- Pipettes and pipette tips
- Deionized or distilled water
- Orbital microplate shaker: 3 mm (0.1118 in) 350 rpm
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times. The shelf life of the components after initial opening is warranted for 4 weeks, store the unused strips and microtiter wells airtight together with the desiccant at 2-8°C in the clip-lock bag, use in the frame provided.
- The reconstituted standards and Controls must be stored at –20°C for up to 4 weeks. For further use, thaw quickly but gently (avoid temperature increase above room temperature and avoid excessive vortexing). Up to 3 of the freeze-thaw cycles did not influence the assay.
- If crystals are observed in the 20X Wash buffer, diluent buffer, warm to RT or 37°C until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- Bring all reagents to room temperature (20- 25°C) before use. Possible precipitations in the buffers have to be resolved before usage by mixing and / or warming.
- When performing the assay, Blank, Standards, Controls and the samples should be pipette as fast as possible (e.g. <15 minutes). To avoid distortions due to differences in incubation times, Antibody Conjugate as well as the succeeding TMB Substrate Solution should be added to the plate in the same order and in the same time interval as the samples. STOP Solution should be added to the plate in the same order as TMB substrate.

- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C for up to 2 years. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA, heparin or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C for up to 2 years. Avoid repeated freeze-thaw cycles.

Note: Hemolytic reactions have to be avoided. Leptin levels show a circadian variation with a peak during the night at about 2 a.m.. This variation together with the influence of food intake needs to be taken into account, when blood samples are collected.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 20X Wash buffer into distilled water to yield 1X Wash buffer. (E.g. 50 ml of 20X Wash buffer + 950 ml of distilled water) The diluted Wash buffer is stable for 4 weeks at 2°C to 8°C.
- Controls: Reconstitute Controls in 500 µl (each) Diluent Buffer. It is recommended to keep reconstituted reagents at room temperature for 15 minutes and then to mix them thoroughly but gently (no foam should result) with a Vortex mixer.
- Standards: Reconstitute Standards in 750 µl (each) Diluent Buffer. It is recommended to keep reconstituted reagents at room temperature for 15 minutes and then to mix them thoroughly but gently (no foam should result) with a Vortex mixer.

  Note: The reconstituted standards and Controls must be stored at –20°C for up to 4 weeks. For further use, thaw quickly but gently (avoid temperature increase above room temperature and avoid excessive vortexing). Up to 3 of the freeze-thaw cycles did not influence the assay.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add **100 µl** of **Dilution Buffer** into all wells.
3. Add **20 µl** of **Dilution Buffer** to the first two wells (blank). Subsequently, add **20 µl** of **each Standard or Control or Sample** to the respective wells.
4. Cover wells and incubate for **1 hour at room temperature** on a microplate shaker (200-350 rpm).
5. Aspirate each well and wash, repeating the process 4 times for a **total 5 washes.** Wash by filling each well with **1× Wash Buffer (300 µl)** using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
6. Add **100 µl** of **Antibody-HRP-Conjugate** into each well. Cover wells and incubate for **30 minutes at room temperature** on a microplate shaker (200-350 rpm).
7. Aspirate and **wash** each well as step 5.
8. Add **100 µl** of **TMB Substrate Reagent** to each well. Incubate for **15 minutes at room temperature** in dark.
9. Add **100 µl** of **Stop Solution** to each well.
10. Read the OD with a microplate reader at **450 nm immediately** (optional:

read at ≥ 590nm as reference wave length). It is recommended read the absorbance within 30 min after adding the stop solution.

CALCULATION OF RESULTS

1. Subtract the blank absorbance value from the standard, control and sample absorbance values. Calculate the average absorbance values for each set of standards, controls and patient samples.

2. Using linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

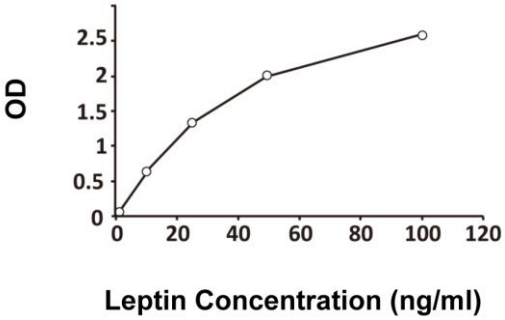
3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

5. For the evaluation of the assay it is required that the absorbance values of the blank should be below 0.25, and the absorbance of standard E should be above 1.00. Samples, which yield higher absorbance values than Standard E, should be re-tested with a dilution.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

Sensitivity

The minimum detectable dose (MDD) of Leptin was 0.25 ng/ml. Based on the undiluted sample the limit of quantification is 1 ng/ml.

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was <6% and inter-assay precision were <12%.

Specificity

This assay is specific for human leptin, No signal was detected in serum of the following species: Horse, Cow, Chicken, Rabbit, Dog, Guinea pig, Sheep, Mouse, Goat, Donkey, Rat, Cat, Pig.

Recovery

108.3%