AssayMax Human Leptin ELISA Kit

Catalog # EL2001-1
Lot #

Introduction

Leptin, a 16-kDa protein secreted from white adipocytes, has been implicated in the regulation of food intake, energy expenditure, and whole-body energy balance in rodents and humans (1). Leptin has been a potential target for treating obesity. The plasma insulin response appears more closely associated with the plasma leptin concentration (2).

Neonatal leptin levels are strongly associated with female gender, birth length, and formula feeding (3). Leptin concentrations were higher in women than in men. In women, serum leptin was the most important predictor of myocardial infarction (MI) (4). In patients with angiographically confirmed coronary atherosclerosis, leptin is a novel predictor of future cardiovascular events independent of other risk factors, including lipid status and CRP (5). Leptin may also play an important role in the pathophysiology of osteoarthritis (OA) (6).

Principal of the Assay

The AssayMax Human Leptin ELISA kit is designed for detection of human leptin in plasma, serum and cell culture supernatants. This assay employs a quantitative sandwich enzyme immunoassay technique which measures leptinin 5 hours. A murine monoclonal antibody specific for leptin has been pre-coated onto a microplate. Leptin in standards and samples is sandwiched by the immobilized antibody and a biotinylated polyclonal antibody specific for leptin, which is recognized by a streptavidin-peroxidase conjugate. All unbound material is then washed away and a peroxidase enzyme substrate is added. The color development is stopped and the intensity of the color is measured.

Caution and Warning

- This kit is for research use only.
- The kit should not be used beyond the expiration date.
- The Stop Solution is an acid solution.

Reagents

- **Leptin Microplate:** A 96-well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody against Leptin.
- **Sealing Tapes:** Each kit contains 3 pre-cut, pressure-sensitive sealing tapes that can be cut to fit the format of the individual assay.
- **Leptin Standard:** Human Leptin in a buffered protein base (6 ng, lyophilized).
- **Biotinylated Leptin Antibody (90x):** A 90-fold biotinylated polyclonal antibody against human leptin (90 µl).
- **Streptavidin-Peroxidase Conjugate (SP Conjugate):** A 100-fold concentrate (120 µl)
- **EIA Diluent Concentrate (10x):** A 10-fold buffered protein base (20 ml).
- **Wash Buffer Concentrate (10x)**: A 10-fold concentrated buffered surfactant (2 x 30 ml).
- **Chromogen Substrate**: A ready-to-use stabilized peroxidase chromogen substrate tetramethylbenzidine (8 ml).
- **Stop Solution**: A 0.5 N hydroxychloric acid (12 ml) to stop the chromogen substrate reaction.

Storage Condition

- Store unopened kit at 2-8^{0}C up to expiration date.
- Opened reagents may be stored for up to 1 month at 2-8^{0}C. Store reconstituted standard at -20^{0}C or below.
- Opened unused strip wells may return to the foil pouch with the desiccant pack, reseal along zip-seal. May be stored for up to 1 month in a vacuum desiccator.

Other Supplies Required

- Microplate reader capable of measuring absorbance at 450 nm
- Pipettes (1-20 µl, 20-200 µl, and multiple channel)
- Deionized or distilled reagent grade water

Sample Collection, Preparation and Storage

- **Plasma:** Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 2,000x g for 10 minutes and assay. Dilute samples 1:25 with EIA Diluent. Store the remaining samples at -20^{0}C or below. Avoid repeated freeze-thaw cycles.
- **Serum:** Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 2,000 x g for 10 minutes. Remove serum and assay. Dilute samples 1:25 into EIA Diluent. Store serum at -20^{0}C or below. Avoid repeated freeze-thaw cycles
- **Cell Culture Supernatants:** Centrifuge cell culture media at 2,000x g for 10 minutes to remove debris. Collect supernatants and assay. Store the remaining samples at -20^{0}C or below. Avoid repeated freeze-thaw cycles

Reagent Preparation

- Freshly dilute all reagents and bring all reagents to room temperature before use. If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved.
- **Standard Curve:** Reconstitute the 6 ng of Human Leptin Standard with 1.5 ml of EIA Diluent to generate a stock solution of 4 ng/ml. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare triplicate standard points by serially diluting the leptin standard solution (4 ng/ml) twofold with equal volume of EIA Diluent to

produce 2, 1, 0.5, 0.25, and 0.125 ng/ml solutions. EIA Diluent serves as the zero standard (0 ng/ml).

Standard Point	Dilution	[Leptin] (ng/ml)
P1	1 part Standard (4 ng/ml)	4.000
P2	1 part P1 + 1 part EIA Diluent	2.000
P3	1 part P2 + 1 part EIA Diluent	1.000
P4	1 part P3 + 1 part EIA Diluent	0.500
P5	1 part P4 + 1 part EIA Diluent	0.250
P6	1 part P5 + 1 part EIA Diluent	0.125
P7	EIA Diluent	0.000

- **EIA Diluent Concentrate (10x):** Dilute EIA Diluent Concentrate 1:10 with reagent grade water.
- **Biotinylated Leptin Antibody (90x):** Spin down the antibody briefly and dilute the desired amount of the antibody 1:90 with EIA Diluent.
- **Wash Buffer Concentrate (10x):** Dilute Wash Buffer Concentrate 1:10 with reagent grade water.
- **SP Conjugate (100x):** Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 1:100 with EIA Diluent.

Assay Procedure

- Prepare all reagents, working standards and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-30^{0}C).
- Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccant inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.
- Add 50 µl of Standard or sample per well. Cover wells and incubate for two hours. Start the timer after the last sample addition.
- Wash five times with 200 µl of Wash Buffer. Invert the plate and decant the contents, and hit it 4-5 times on absorbent paper towel to complete remove liquid at each step.
- Add 50 µl of Biotinylated Leptin Antibody to each well and incubate for two hours.
- Wash five times with 200 µl of Wash Buffer as above.
- Add 50 µl of Streptavidin-Peroxidase Conjugate per well and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.
- Wash five times with 200 µl of Wash Buffer as above.
- Add 50 µl of Chromogen Substrate per well and incubate for about 10 minutes or till the optimal blue color density develops. Gently tap the plate to ensure thorough mixing and break the bubbles in the well with pipette tip.
- Add 50 µl of Stop Solution to each well. The color will change from blue to yellow.
- Read the absorbance on a microplate reader at a wavelength of 450 nm **immediately**. Please note that some unstable black particles may be generated at high concentration points after stopping the reaction for about 10 minutes, which will reduce the readings.

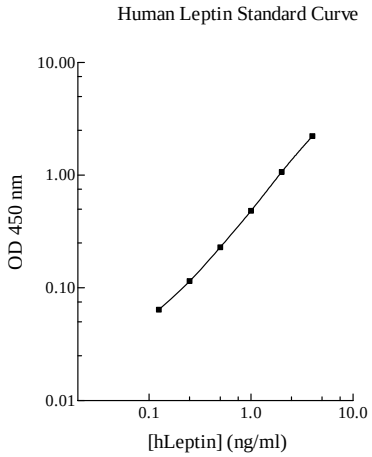
Data Analysis

- Calculate the mean value of the triplicate readings for each standard and sample.

- To generate a standard curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean 450 nm absorbance on the y-axis. The best-fit line can be determined by regression analysis using 4-parameter or log-log curve fit.
- Determine the unknown sample concentration from the Standard Curve and multiply the plasma or serum mean value by the dilution factor of 25.

Standard Curve

- The curve is provided for illustration only. A standard curve should be generated each time the assay is performed.



Performance Characteristics

- The minimum detectable level of leptin is typically < 100 pg/ml.
- Intra-assay and inter-assay coefficients of variation were 5.5 % and 7.4% respectively.
- No significant cross-reactivity or interference was observed.

References

1. Houseknecht KL *et. al* (1998) *J Anim Sci.* 76(5):1405-20.
2. Abbasi F *et. al.* (2000) *Metabolism.* 49(4):544-7
3. Petridou E *et. al* (2005) *Clin Endocrinol (Oxf).* 62(3):366-71
4. Wallerstedt SM *et. al* (2004) *Blood Press* 13(4):243-6
5. Wolk R *et. al* (2004) *J Am Coll Cardiol.* 44(9):1819-24
6. Dumond H *et. al.* (2003) *Arthritis Rheum.* 48(11):3118-29

Revision 2.2