KAMIYA BIOMEDICAL COMPANY

Human Leptin ELISA

For the quantitative determination of human leptin in serum, plasma, cell culture supernatants, body fluid and tissue homogenate

Cat. No. KT-53225

For Research Use Only. Not for use in diagnostic procedures.

Product Information

Human Leptin ELISA

Cat. No. KT-53225

INTENDED USE

This Leptin ELISA kit is a 1.5 hour solid-phase ELISA designed for the quantitative determination of Human Leptin. This ELISA kit is for research use only, not for therapeutic or diagnostic applications!

PRINCIPLE

Leptin ELISA kit applies the competitive enzyme immunoassay technique utilizing a monoclonal anti-Leptin antibody and a Leptin-HRP conjugate. The assay sample and buffer are incubated together with Leptin-HRP conjugate in pre-coated plate for one hour. After the incubation period, the wells are decanted and washed five times. The wells are then incubated with a substrate for HRP enzyme. The product of the enzyme-substrate reaction forms a blue colored complex. Finally, a stop solution is added to stop the reaction, which will then turn the solution yellow. The intensity of color is measured spectrophotometrically at 450 nm in a microplate reader. The intensity of the color is inversely proportional to the concentration since Leptin from samples and Leptin-HRP conjugate compete for the anti-Leptin antibody binding site. Since the number of sites is limited, as more sites are occupied by Leptin from the sample, fewer sites are left to bind Leptin-HRP conjugate. A calibration curve is plotted relating the intensity of the color (O.D.) to the concentration of calibrators. The Leptin concentration in each sample is interpolated from this calibration curve.

COMPONENTS

Reagents	Quantity
Microtiter Plate	96 wells
Calibrator 1 (0 ng/mL)	1
Calibrator 2 (0.5 ng/mL)	1
Calibrator 3 (1 ng/mL)	1
Calibrator 4 (2.5 ng/mL)	1
Calibrator 5 (5 ng/mL)	1
Calibrator 6 (10 ng/mL)	1
Enzyme Conjugate	1 x 6 mL
Substrate A	1 x 6 mL
Substrate B	1 x 6 mL
Stop Solution	1 x 6 mL
Wash Buffer (100X concentrate)	1 x 10 mL
Balance Solution	1 x 3 mL

Note: The balance solution is used only when the sample is cell culture supernatants, body fluid and tissue homogenate; if the sample is serum or plasma, then the balance solution is a superfluous reagent.

STORAGE

All reagents provided are stored at 4°C. Refer to the expiration date on the label.

SAMPLE COLLECTION AND STORAGE

Serum

Use a serum separator tube and allow samples to clot for 2 hours at room temperature or overnight at 4℃. Centrifuge at approximately 1,000 x g (or 3,000 rpm) for 15 mintues. Remove serum and assay immediately or aliquot and store samples at -20℃ or -80℃.

Plasma

Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1,000 x g (or 3,000 rpm) at 4℃ within 30 minutes of collection. Assay immediately or aliquot and store samples at -20℃ or -80℃.

Tissue homogenates

The preparation of tissue homogenates will vary depending upon tissue type. For this assay, tissues were rinsed in ice-cold PBS (0.02 mol/L, pH 7.0-7.2) to remove excess blood thoroughly and weighed before homogenization. Minced the tissues to small pieces and homogenized them in a certain amount of PBS with a glass homogenizer on ice. The resulting suspension was subjected to ultrasonication or to two freeze-thaw cycles to further break the cell membranes. After that, the homogenates were centrifugated for 15 minutes at 1,500 x g (or 5,000 rpm). Remove the supernate and assay immediately or aliquot and store samples at -20℃ or -80℃.

Cell lysates

Cells should be lysed according to the following directions.

1. Adherent cells should be detached with trypsin and then collected by centrifugation. Suspension cells can be collected by centrifugation directly.
2. Wash cells three times in PBS.
3. Cells were resuspended in PBS and subjected to ultrasonication for 3 times. Alternatively, freeze cells at -20℃. Thaw cells with gentle mixing. Repeat the freeze/thaw cycle for 3 times.
4. Centrifuge at 1,000 x g (or 3,000 rpm) for 15 minutes at 4℃ to remove cellular debris.
5. Assay immediately or store samples at -20℃ or -80℃.

Cell culture supernatants and other body fluids

Centrifuge cell culture media at 1,000 x g (or 3,000 rpm) for 15 minutes to remove debris. Assay immediately or store samples at -20℃ or -80℃.

NOTE:

1. Samples should be aliquoted and must be stored at -20℃ (less than 3 months) or -80℃ (less than 6 months) to avoid loss of bioactivity and contamination. If samples are to be run within 24 hours, they may be stored at 4℃. Avoid repeated freeze-thaw cycles.
2. Prior to assay, the frozen sample should be brought to room temperature slowly and mixed gently.
3. Tissue or cell extraction samples prepared by chemical lysis buffer may cause unexpected ELISA results due to the impacts of certain chemicals.
4. Samples containing a visible precipitate must be clarified prior to use in the assay. Care should be taken to minimize hemolysis. Do not use grossly hemolyzed or lipemic specimens.
5. Do not use heat-treated specimens.

MATERIALS REQUIRED BUT NOT SUPPLIED

1. Precision pipettors and disposable tips to deliver 10-1,000 µL. A multi-channel pipette is desirable for large assays.
2. 100 mL and 1 liter graduated cylinders.
3. Distilled or deionized water.
4. Tubes to prepare sample dilutions.
5. Absorbent paper.