Datasheet for ABIN625035

Leptin ELISA Kit

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Overview

Quantity:	96 tests
Target:	Leptin (LEP)
Reactivity:	Human
Method Type:	Sandwich ELISA
Detection Range:	2-400 pg/mL
Minimum Detection Limit:	2 pg/mL
Application:	ELISA

Product Details

Purpose:	Human Leptin ELISA Kit for cell culture supernatants, plasma, and serum samples.
Sample Type:	Plasma, Cell Culture Supernatant, Serum
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Specificity:	The Leptin ELISA kit shows no cross-reactivity with any of the cytokines tested: Human Angiogenin, BDNF, BLC, ENA-78, FGF-4, IL-1 alpha, IL-1 beta, IL-2, IL-3, IL-4, IL-5, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12 p70, IL-12 p40, IL-13, IL-15, I-309, IP-10, G-CSF, GM-CSF, IFN-gamma, MCP- 1, MCP-2, MCP-3, MDC, MIP-1 alpha, MIP-1 beta, MIP-1 delta, PARC, PDGF, RANTES, SCF, TARC, TGF-beta, TIMP-1, TIMP-2, TNF-alpha, TNF-beta, TPO, VEGF.
Sensitivity:	< 2 pg/mL
Characteristics:	• Strip plates and additional reagents allow for use in multiple experiments

Product Details

	• Quantitative protein detection • Establishes normal range • The best products for confirmation of antibody array data
Components:	• Pre-Coated 96-well Strip Microplate • Wash Buffer • Stop Solution • Assay Diluent(s) • Lyophilized Standard • Biotinylated Detection Antibody • Streptavidin-Conjugated HRP • TMB One-Step Substrate
Material not included:	• Distilled or deionized water • Precision pipettes to deliver 2 µL to 1 µL volumes • Adjustable 1-25 µL pipettes for reagent preparation • 100 µL and 1 liter graduated cylinders • Tubes to prepare standard and sample dilutions • Absorbent paper • Microplate reader capable of measuring absorbance at 450nm • Log-log graph paper or computer and software for ELISA data analysis

Target Details

Target:	Leptin (LEP)
Alternative Name:	Leptin (LEP Products)
Background:	Leptin is a secreted protein of 16 kDa. Human and murine leptin show approximately 84 percent identity at the protein level. Leptin inhibits food intake and stimulates energy expenditure. Leptin also has thermogenic actions and regulates enzymes of fatty acid oxidation. Studies indicate that human obesity may be associated with leptin receptor deficiencies rather than constituting a problem with leptin itself. The Human Leptin ELISA (Enzyme-Linked Immunosorbent Assay) kit is an in vitro enzyme-linked immunosorbent assay for the quantitative measurement of human Leptin in serum, plasma, cell culture supernatants and urine. This assay employs an antibody specific for human Leptin coated on a 96-well plate. Standards and samples are pipetted into the wells and Leptin present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti-human Leptin antibody is added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin is pipetted to the wells. The wells are again washed, a TMB substrate solution is added to the wells and color develops in proportion to the amount of Leptin bound. The Stop

Target Details

	Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm. Reproducibility: Intra-Assay: CV<10% Inter-Assay: CV<12%.
Gene ID:	3952
UniProt:	P41159
Pathways:	JAK-STAT Signaling, AMPK Signaling, Hormone Transport, Peptide Hormone Metabolism, Hormone Activity, Negative Regulation of Hormone Secretion, Regulation of Carbohydrate Metabolic Process, Feeding Behaviour, Monocarboxylic Acid Catabolic Process

Application Details

Application Notes:	Recommended Dilution for serum and plasma samples20 - 50 fold
Sample Volume:	100 µL
Plate:	Pre-coated
Protocol:	1. Prepare all reagents, samples and standards as instructed in the manual. 2. Add 100 µL of standard or sample to each well. 3. Incubate 2.5 h at RT or O/N at 4 °C. 4. Add 100 µL of prepared biotin antibody to each well. 5. Incubate 1 h at RT. 6. Add 100 µL of prepared Streptavidin solution to each well. 7. Incubate 45 min at RT. 8. Add 100 µL of TMB One-Step Substrate Reagent to each well. 9. Incubate 30 min at RT. 10. Add 50 µL of Stop Solution to each well. 11. Read at 450 nm immediately.
Reagent Preparation:	1. Bring all reagents and samples to room temperature (18 - 25 °C) before use. 2. Sample dilution: If your samples need to be diluted, Assay Diluent A (Item D) should be used for dilution of serum/plasma samples. 1x Assay Diluent B (Item E) should be used for dilution of culture supernatants and urine. Suggested dilution for normal serum/plasma: 20-50 fold*. *Please note that levels of the target protein may vary between different specimens. Optimal dilution factors for each sample must be determined by the investigator. 3. Assay Diluent B should be diluted 5-fold with deionized or distilled water. 4. Preparation of standard: Briefly spin the vial of Item C. Add 440 µL Assay Diluent A (for serum/plasma samples) or 1x Assay Diluent B (for cell culture medium and urine) into Item C vial to prepare a 220 ng/mL standard. Dissolve the powder thoroughly by a gentle mix. Add 2 µL Leptin standard from the vial of Item C, into a tube with 1098 µL Assay Diluent A (for

	serum/plasma samples) or 1x Assay Diluent B (for cell culture medium and urine) to prepare a 400 pg/mL stock standard solution. Pipette 300 µL Assay Diluent A or 1x Assay Diluent B into each tube. Use the stock standard solution to produce a dilution series . Mix each tube thoroughly before the next transfer. Assay Diluent A or 1x Assay Diluent B serves as the zero standard (0 pg/mL). 200 µL 200 µL 200 µL 200 µL 200 µL 200myl 2 µL standard + 1098 µL 400 160 64 25.6 10.24 4.10 1.64 0 pg/mL pg/mL pg/mL pg/mL pg/mL pg/mL pg/mL pg/mL 5. If the Wash Concentrate (20x) (Item B) contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute 20 ml of Wash Buffer Concentrate into deionized or distilled water to yield 400 ml of 1x Wash Buffer. 6. Briefly spin the Detection Antibody vial (Item F) before use. Add 100 µL of 1x Assay Diluent B into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently (the concentrate can be stored at 4 °C for 5 days). The detection antibody concentrate should be diluted 80-fold with 1x Assay Diluent B and used in step 4 of Part VI Assay Procedure. 7. Briefly spin the HRP-Streptavidin concentrate vial (Item G) and pipette up and down to mix gently before use. HRP-Streptavidin concentrate should be diluted 160-fold with 1x Assay Diluent B. For example: Briefly spin the vial (Item G) and pipette up and down to mix gently . Add 100 µL of HRP-Streptavidin concentrate into a tube with 16 ml 1x Assay Diluent B to prepare a final 160 fold diluted HRP-Streptavidin solution (don't store the diluted solution for next day use). Mix well.
Assay Procedure:	1. Bring all reagents and samples to room temperature (18 - 25 °C) before use. It is recommended that all standards and samples be run at least in duplicate. 2. Add 100 µL of each standard (see Reagent Preparation step 2) and sample into appropriate wells. Cover well and incubate for 2.5 hours at room temperature or over night at 4 °C with gentle shaking. 3. Discard the solution and wash 4 times with 1x Wash Solution. Wash by filling each well with Wash Buffer (300 myl) using a multi-channel Pipette or autowasher. 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. 4. Add 100 µL of 1x prepared biotinylated antibody (Reagent Preparation step 6) to each well. Incubate for 1 hour at room temperature with gentle shaking. 5. Discard the solution. Repeat the wash as in step 6. Add 100 µL of prepared Streptavidin solution (see Reagent Preparation step 7) to each well. Incubate for 45 minutes at room temperature with gentle shaking. 7. Discard the solution. Repeat the wash as in step 8. Add 100 µL of TMB One-Step Substrate Reagent (Item H) to each well. Incubate for 30

Application Details

	minutes at room temperature in the dark with gentle shaking. 9. Add 50 µL of Stop Solution (Item I) to each well. Read at 450 nm immediately.
Calculation of Results:	Calculate the mean absorbance for each set of duplicate standards, controls and samples, and subtract the average zero standard optical density. Plot the standard curve on log-log graph paper or using Sigma plot software, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit straight line through the standard points. Typical Data: These standard curves are for demonstration only. A standard curve must be run with each assay. Assay Diluent A Human Leptin concentration (pg/mL) 1 10 100 1000 O D =4 50 n m 0.01 0.1 1 10 Assay Diluent B Human Leptin concentration (pg/mL) 1 10 100 1000 O D =4 50 n m 0.01 0.1 1 10 Sensitivity: The minimum detectable dose of Leptin is typically less than 2 pg/mL. Recovery: Recovery was determined by spiking various levels of human Leptin into human serum, plasma and cell culture media. Mean recoveries are as follows: Sample Type Average % Recovery Range (%) Serum 94.49 83-103 Plasma 93.85 82-102 Cell culture media 95.42 84-104 Linearity: Sample Type Serum Plasma Cell Culture Media 1:2 Average % of Expected 92 91 93 Range (%) 82-102 81-101 83-103 1:4 Average % of Expected 94 95 92 Range (%) 83-103 82-102 83-102 Reproducibility: Intra-Assay: CV<10 % Inter-Assay: CV<12 %
Assay Precision:	Intra-Assay: CV< 10 % Inter-Assay: CV< 12 %
Restrictions:	For Research Use only

Handling

Handling Advice:	Avoid repeated freeze-thaw cycles.
Storage:	-20 °C
Storage Comment:	The entire kit may be stored at -20°C for up to 1 year from the date of shipment. Avoid repeated freeze-thaw cycles. The kit may be stored at 4°C for up to 6 months. For extended storage, it is recommended to store at -80°C.
Expiry Date:	6 months

Publications

Product cited in:	Joseph, Kumar: "Identifying clues to molecular etiology of multiple sclerosis in South Indian patients." in: **Multiple sclerosis and related disorders**, Vol. 5, pp. 7-11, (2016) (PubMed).

Publications

Jørgensen, Nellemann, Wohlfahrt-Veje, Jensen, Main, Andersen: "Interaction between paraoxonase 1 polymorphism and prenatal pesticide exposure on metabolic markers in children using a multiplex approach." in: **Reproductive toxicology (Elmsford, N.Y.)**, Vol. 51, pp. 22-30, (2015) (PubMed).

Lin, Huybrechts, Vereecken, Mouratidou, Valtueña, Kersting, González-Gross, Bolca, Wärnberg, Cuenca-García, Gottrand, Toti, Gomez-Martínez, Grammatikaki, Labayen, Moreno, Sjöström, Van Camp, Roccaldo et al.: "Dietary fiber intake and its association with indicators of adiposity and serum biomarkers in European adolescents: the HELENA study. ..." in: **European journal of nutrition**, Vol. 54, Issue 5, pp. 771-82, (2015) (PubMed).

Ciaffi, Cavassini, Genne, Delhumeau, Spycher Elbes, Hill, Wandeler, Fehr, Stoeckle, Schmid, Hirschel, Montecucco, Calmy: "Switch to etravirine for HIV-positive patients receiving statin treatment: a prospective study." in: **European journal of clinical investigation**, Vol. 45, Issue 7, pp. 720-30, (2015) (PubMed).

Nobili, Alisi, Cutrera, Carpino, De Stefanis, DOria, De Vito, Cucchiara, Gaudio, Musso: "Altered gut-liver axis and hepatic adiponectin expression in OSAS: novel mediators of liver injury in paediatric non-alcoholic fatty liver." in: **Thorax**, Vol. 70, Issue 8, pp. 769-81, (2015) (PubMed).

There are more publications referencing this product on: Product page

Images

ELISA

Image 1.