

Datasheet for ABIN625035

Leptin ELISA Kit

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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 μ L 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 μ L 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 μ L 900 μ L 200 μ L 200 μ L 200 μ L 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 μ L 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 $100~\mu\text{L}$ 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 μ L 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 μ L 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 μ L 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

perature in the dark with gentle shaking. colution (Item I) to each well. Read at 450 nm immediately. sorbance for each set of duplicate standards, controls and samples, and ero standard optical density. Plot the standard curve on log-log graph plot software, with standard concentration on the x-axis and absorbance e best-fit straight line through the standard points. andard curves are for demonstration only. A standard curve must be run y Diluent A Human Leptin concentration (pg/mL) 1 10 100 1000 0 D =4
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assay Diluent B Human Leptin concentration (pg/mL) 1 10 100 1000 O D
um detectable dose of Leptin is typically less than 2 pg/mL.
as determined by spiking various levels of human Leptin into human
Il culture media. Mean recoveries are as follows: Sample Type Average %
erum 94.49 83-103 Plasma 93.85 82-102 Cell culture media 95.42 84-
e Serum Plasma Cell Culture Media 1:2 Average % of Expected 92 91 93
101 83-103 1:4 Average % of Expected 94 95 92 Range (%) 83-103 82-
assay: CV<10 % Inter-Assay: CV<12 %
Inter-Assay: CV< 12 %
thaw cycles.
stored at -20°C for up to 1 year from the date of shipment. Avoid repeated
e kit may be stored at 4°C for up to 6 months. For extended storage, it is
e at -80°C.
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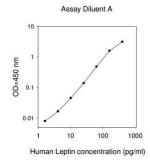
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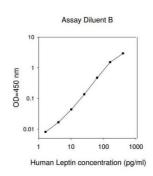
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There are more publications referencing this product on: Product page

Images





ELISA

Image 1.