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Leptin ELISA Kit





Publication



Overview

Quantity:	96 tests
Target:	Leptin (LEP)
Reactivity:	Rabbit
Method Type:	Competition ELISA
Detection Range:	15.6 pg/mL - 1000 pg/mL
Minimum Detection Limit:	15.6 pg/mL
Application:	ELISA
Product Details	
Purpose:	For the quantitative determination of rabbit leptin(LEP) concentrations in serum, plasma, tissue homogenates.
Sample Type:	Plasma, Serum, Tissue Homogenate
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Specificity:	This assay has high sensitivity and excellent specificity for detection of rabbit LEP. No significant cross-reactivity or interference between rabbit LEP and analogues was observed. Note: Limited by current skills and knowledge, it is impossible for us to complete the cross-reactivity detection between rabbit LEP and all the analogues, therefore, cross reaction may still exist.
Sensitivity:	3.9 pg/mL

Product Details

Components:

- Assay plate
- Standard
- HRP-conjugate (100 x concentrate)
- · Sample Diluent
- HRP-conjugate Diluent
- Wash Buffer (25 x concentrate)
- TMB Substrate
- · Stop Solution
- · Adhesive Strip
- · Stop Solution
- Adhesive Strip

Target Details

Target:	Leptin (LEP)
Alternative Name:	Leptin,LEP (LEP Products)
Background:	Abbreviation: LEP
	Alias: N/A
UniProt:	B0KZL0
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:	Optimal working dilution should be determined by the investigator.
Sample Volume:	50 μL
Assay Time:	1 - 4.5 h
Plate:	Pre-coated
Protocol:	Prepare reagents, samples and standards as instructed.
	2. Set a Blank well without any solution.
	3. Add 50 µL standard or sample to each well.
	4. Add 50 μL HRP-conjugate (1x) to each well (Not to Blank well).
	5. Incubate 1 hour at 37 °C
	6. Aspirate and wash 5 times.
	7. Add 90 μL of TMB Substrate to each well. Incubate for 20 minutes at 37 °C. Protect from

light.

8. Add 50 µL Stop Solution to each well. Read at 450 nm within 5 minutes.

Reagent Preparation:

- 1. HRP-conjugate (1x) Centrifuge the vial before opening. HRP-conjugate requires a 100-fold dilution. A suggested 100-fold dilution is 10 μ L of HRP-conjugate + 990 μ L of HRP-conjugate Diluent.
- 2. Wash Buffer(1x)- If crystals have formed in the concentrate, warm up to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 mL of Wash Buffer Concentrate (25 x) into deionized or distilled water to prepare 500 mL of Wash Buffer (1 x).
- 3. Standard Centrifuge the standard vial at 6000-10000rpm for 30s before opening. Dilute the Standard(10x) with Sample Diluent. A suggested 10-fold dilution is 30 µL of Standard(10x) + 270 µL of Sample Diluent. This diluted Standard (S7) serves as the high standard (1000 pg/mL). Do not substitute other diluents. Mix the standard to ensure complete dilution and allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 150 µL of Sample Diluent into each tube (S0-S6). Use the diluted Standard (S7) solution to produce a 2-fold dilution series (below). Mix each tube thoroughly before the next transfer. Sample Diluent serves as the zero standard (0 pg/mL).

Note:

- Kindly use graduated containers to prepare the reagent. Please don't prepare the reagent directly in the Diluent vials provided in the kit.
- Bring all reagents to room temperature (18-25 °C) before use for 30 min.
- Prepare fresh standard for each assay. Use within 4 hours and discard after use.
- · Making serial dilution in the wells directly is not permitted.
- Distilled water is recommended to be used to make the preparation for reagents.
 Contaminated water or container for reagent preparation will influence the detection result.

Sample Preparation:

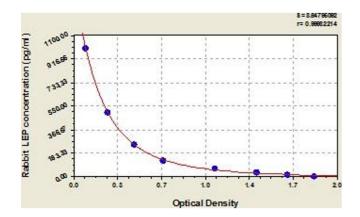
- It is recommended to use fresh samples without long storage, otherwise protein degradation and denaturationmay occur in these samples, leading to false results. Samples should therefore be stored for a short periodat 2 8 °C or aliquoted at -20 °C (≤1 month) or -80 °C (≤ 3 months). Repeated freeze-thawcycles should be avoided. Prior to assay, the frozen samples should be slowly thawed and centrifuged toremove precipitates.
- If the sample type is not specified in the instructions, a preliminary test is necessary to determine compatibility with the kit.
- If a lysis buffer is used to prepare tissue homogenates or cell culture supernatant, there is a possibility of causing a deviation due to the introduced chemical substance. The recommended dilution factor is for reference only.
- Please estimate the concentration of the samples before performing the test. If the values are not in therange of the standard curve, the optimal sample dilution for the particular experiment has to be determined. Samples should then be diluted with PBS (pH =7.0-7.2).

Note:

Recommend to dilute the serum or plasma samples with Sample Diluent(1:200) before test. The suggested 200-fold dilution can be achieved by adding $5 \,\mu L$ sample to $45 \,\mu L$ of Sample

Application Details

	Diluent. Complete the 200-fold dilution by adding15 μL of this solution to 285 μL of Sample
	Diluent. The recommended dilution factor is for reference only. The optimal dilution factor
	should be determined by users according to their particular experiments.
Assay Precision:	Intra-assay Precision (Precision within an assay): CV%<8% Three samples of known
	concentration were tested twenty times on one plate to assess.
	Inter-assay Precision (Precision between assays): CV%<10% Three samples of known
	concentration were tested in twenty assays to assess.
Restrictions:	For Research Use only
Handling	
Storage:	4 °C,-20 °C
Storage Comment:	Unopened kit Store at 2 - 8°C. Do not use the kit beyond the expiration date. May be stored for
	up to 1 month at 2 - 8°C. Coated assay Try to keep it in a sealed aluminum foil bag, plate and
	avoid the damp. Standard May be stored for up to 1 month at 2 - 8° C. If don't make recent use,
	better keep it store at HRP-conjugate -20°C. Opened kit HRP-conjugate Diluent Sample Diluent
	May be stored for up to 1 month at 2 - 8°C. Wash Buffer TMB Substrate Stop Solution *Provided
	this is within the expiration date of the kit.
Expiry Date:	6 months
Publications	
Product cited in:	Xu, Ge, Qin, Peng, Zhang, Liu, Liang, Wang, Chen, Li: "Acute, but not chronic, stress increased the
	plasma concentration and hypothalamic mRNA expression of NUCB2/nesfatin-1 in rats." in:
	Neuropeptides, (2015) (PubMed).
	Ge, Xu, Qin, Peng, Zhang, Liu, Liang, Wang, Chen: "Depression-like Behavior Induced by Nesfatin-
	1 in Rats: Involvement of Increased Immune Activation and Imbalance of Synaptic Vesicle
	Proteins." in: Frontiers in neuroscience , Vol. 9, pp. 429, (2015) (PubMed).
	Haghshenas, Jafari, Ravasi, Kordi, Gilani, Shariatzadeh, Hedayati, Rahimi: "The effect of eight
	weeks endurance training and high-fat diet on appetite-regulating hormones in rat plasma." in:
	Iranian journal of basic medical sciences, Vol. 17, Issue 4, pp. 237-43, (2014) (PubMed).



ELISA

Image 1. Typical Standard Curve