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APA084Mu01 100µg Active Leptin (LEP) Organism Species: *Mus musculus (Mouse) Instruction manual*

FOR RESEARCH USE ONLY NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

13th Edition (Revised in Aug, 2023)

[PROPERTIES]

Source: Prokaryotic expression. Host: *E. coli* Residues: Val22~Cys167 Tags: N-terminal His-tag Purity: >90% Endotoxin Level: <1.0EU per 1µg (determined by the LAL method). Buffer Formulation: PBS, pH7.4, containing 0.01% SKL, 5%Trehalose . Original Concentration: 200µg/mL Applications: Cell culture; Activity Assays. (May be suitable for use in other assays to be determined by the end user.) Predicted isoelectric point: 8.4 Predicted Molecular Mass: 21.7kDa Accurate Molecular Mass: 25kDa as determined by SDS-PAGE reducing conditions.

[<u>USAGE</u>]

Reconstitute in 10mM PBS (pH7.4) to a concentration of 0.1-1.0 mg/mL. Do not vortex.

[STORAGE AND STABILITY]

Storage: Avoid repeated freeze/thaw cycles.

Store at 2-8°C for one month.

Aliquot and store at -80°C for 12 months.

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Stability Test: The thermal stability is described by the loss rate. The loss rate was determined by accelerated thermal degradation test, that is, incubate the protein at 37°C for 48h, and no obvious degradation and precipitation were observed. The loss rate is less than 5% within the expiration date under appropriate storage condition.

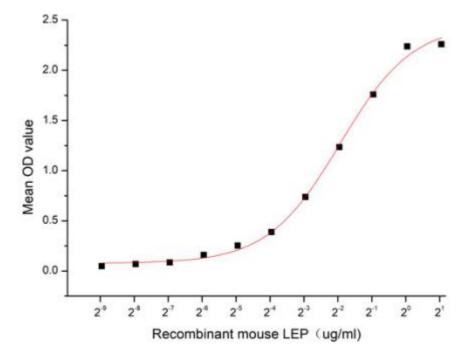
[SEQUENCE]

VPIQKVQDD TKTLIKTIVT RINDISHTQS VSAKQRVTGL DFIPGLHPIL SLSKMDQTLA VYQQVLTSLP SQNVLQIAND LENLRDLLHL LAFSKSCSLP QTSGLQKPES LDGVLEASLY STEVVALSRL QGSLQDILQQ LDVSPEC

[ACTIVITY]

Leptin is a hormone made by adipose cells that helps to regulate energy balance inhibiting hunger. Many of leptin's effects are mediated through by neuropeptide-containing neurons and neuropeptide receptors in the hypothalamus. Although regulation of fat stores is deemed to be the primary function of leptin, it also plays a role in other physiological processes, as evidenced by its multiple sites of synthesis other than fat cells, and the multiple cell types beside hypothalamic cells that have leptin receptors. A functional binding ELISA assay was conducted to detect the interaction of recombinant mouse LEP and recombinant human LEPR. Briefly, LEP were diluted serially in PBS, with 0.01% BSA (pH 7.4). Duplicate samples of 100 ul were then transferred to LEPR-coated microtiter wells and incubated for 2h at 37 °C. Wells were washed with PBST and incubated for 1h with anti-LEP pAb, then aspirated and washed 3 times. After incubation with HRP labelled secondary antibody, wells were aspirated and washed 3 times. With the addition of substrate solution, wells were incubated 15-25 minutes at 37 $^{\circ}$ C. Finally, add 50 µL stop solution to the wells and read at 450 nm immediately. The binding activity of of LEP and LEPR was shown in Figure 1, Cloud-Clone Corp.

the EC50 for this effect is 0.26 ug/mL.





[IDENTIFICATION]

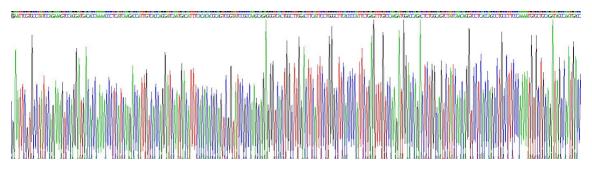


Figure 2. Gene Sequencing (extract)

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	kDa 70
	44
	33
_	26
	22
	18
	14
	10

Figure 3. SDS-PAGE

Sample: Active recombinant LEP, Mouse

[IMPORTANT NOTE]

The kit is designed for research use only, we will not be responsible for any issue if the kit was used in clinical diagnostic or any other procedures.