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APA085Ra01 100µg Active Leukemia Inhibitory Factor (LIF) Organism Species: *Rattus norvegicus (Rat) Instruction manual*

FOR RESEARCH USE ONLY NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

1st Edition (Apr, 2016)

[PROPERTIES]

Source: Prokaryotic expression. Host: *E. coli* Residues: Leu25~Phe202 Tags: N-terminal His-tag Purity: >94% Endotoxin Level: <1.0EU per 1µg (determined by the LAL method). Buffer Formulation: PBS, pH7.4, containing 0.01% SKL, 5% Trehalose. Applications: Cell culture; Activity Assays. (May be suitable for use in other assays to be determined by the end user.) Predicted isoelectric point: 8.9 Predicted Molecular Mass: 23.3kDa 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.

[<u>SEQUENCE</u>]

LPITPVNATCAIRHPCHGNLMNQIKSQLAQLNGSANALFISYYTAQGEPFPNNV DKLCAPNMTDFPPFHANGTEKTKLVELYRMVTYLGASLTNITWDQKNLNPTAV SLQIKLNATTDVMRGLLSSVLCRLCNKYHVGHVDVPCVPDNSSKEAFQRKKLG CQLLGTYKQVISVLAQAF

[ACTIVITY]

Leukemia inhibitory factor (LIF), is an interleukin 6 class cytokine that affects cell growth by inhibiting differentiation. LIF as a cytokine also has another fuction including: the growth promotion and cell differentiation of different types of target cells. influence on bone metabolism, cachexia, neural development, embryogenesis and inflammation. To evaluate the activity of recombinant rat LIF, the K562 cells were were seeded into triplicate wells of 96-well plates at a density of 5,000 cells/well with5% serum standard 1640 which contains various concentrations of recombinant rat LIF. After incubated for 72h, cells were observed by inverted microscope and cell proliferation was measured by Cell Counting Kit-8 (CCK-8). Briefly, 10 µl of CCK-8 solution was added to each well of the plate, then the absorbance at 450 nm was measured using a microplate reader after incubating the plate for 2-4 hours at 37 °C. After incubation with LIF for72h, proliferative inhibition of K562 cells observed by inverted microscope was shown in Figure1. Cell viability was assessed by CCK-8(Cell Counting Kit-8) assay after incubation with recombinant LIF for72h. The result was shown in Figure 2. It was obvious that LIF significantly inhibit cell viability of K562 cells. The ED50 is 1.44µg/ml.

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(A) Unstimulated k562cells cultured in 1640 for 72h;(B) K562 cells cultured in 1640, stimulated with 1.25µg/ml LIF for 72h.





[IDENTIFICATION]

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	kDa 70
	44
	33
-	26
	22
	18
	14
	10

Figure 3. SDS-PAGE

Sample: Active recombinant LIF, Rat



Figure 4. Western Blot

Sample: Recombinant LIF, Rat;

Antibody: Rabbit Anti- Rat LIF Ab (PAA085Ra01)

[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.