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APA080Rb01 100µg Active Interleukin 8 (IL8) Organism Species: Oryctolagus cuniculus (Rabbit) *Instruction manual* 

### FOR IN VITRO USE AND RESEARCH USE ONLY NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

1st Edition (Apr, 2016)

## [PROPERTIES]

Source: Prokaryotic expression.

Host: E. coli

Residues: Ala23~Ser101

Tags: N-terminal His-tag

**Purity: >95%** 

**Buffer Formulation:** 20mM Tris, 150mM NaCl, pH8.0, containing 0.05% sarcosyl and 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: 9.1

Predicted Molecular Mass: 12.5kDa

Accurate Molecular Mass: 14kDa as determined by SDS-PAGE reducing conditions.

## [ <u>USAGE</u> ]

Reconstitute in 20mM Tris, 150mM NaCl (pH8.0) 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]

### AVLTRIGT ELRCQCIKTH STPFHPKFIK ELRVIESGPH CANSEIIVKL VDGRELCLDP KEKWVQKVVQ IFLKRAEQQE S

# [ACTIVITY]

Interleukin 8 (IL8 or chemokine (C-X-C motif) ligand 8, CXCL8) is a chemokine produced by macrophages and other cell types such as epithelial cells, airway smooth muscle cells and endothelial cells. Thus, chemotaxis assay used 24-well microchemotaxis system was undertaken to detect the chemotactic effect of IL8 on the human T-lymphocyte leukemia cell line Jurkat. Briefly, Jurkat cells were seeded into the upper chambers (100uL cell suspension, 10<sup>6</sup>cells/ml in RPMI 1640 with FBS free) and IL8 (0.1ng/mL, 1ng/mL and 10ng/mL diluted separately in serum free RPMI 1640 ) was added in lower chamber with a polycarbonate filter (8um pore size) used to separate the two compartments. After incubation at 37  $^\circ\!\!\mathbb{C}$ with 5% CO<sub>2</sub> for 1h, the filter was removed, then cells in low chamber were observed by inverted microscope at low magnification (×100) and the number of migrated cells were counted at high magnification (×400) randomly (five fields for each filter). Result shows IL8 is able to induce migration of Jurkat cells. The migrated Jurkat cells in low chamber at low magnification (×100) were shown in Figure 1. Five fields of each chamber were randomly chosen, and the migrated cells were counted at high magnification (×400). Statistical results were shown in Figure 2. The optimum chemotaxis of IL8 occurs at 1~10ng/mL.

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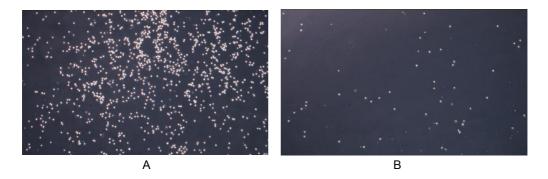


Figure 1.The chemotactic effect of IL8 on Jurkat cells.

(A) Jurkat cells were seeded into the upper chambers and serum free RPMI 1640 with 1ng/mL IL8 was added in lower chamber, then cells in lower chamber were observed at low magnification (×100) after incubation for 1h;

(B) Jurkat cells were seeded into the upper chambers and serum free RPMI 1640 without IL8 was added in lower chamber, then cells in lower chamber were observed at low magnification (×100) after incubation for 1h.

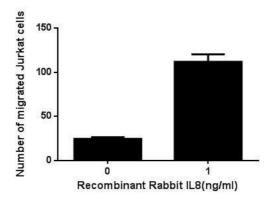
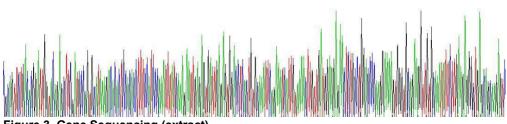


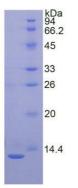
Figure 2. The chemotactic effect of IL8 on Jurkat cells.

# [ IDENTIFICATION ]



A VITRIGTEIRCQCIKTHSTPFHPKFIKELRVIESGPHCANSETIVKLVDGREICLDPKEKVVQKVVQIFLKRAEQQES

### Figure 3. Gene Sequencing (extract)



#### Figure 4. SDS-PAGE

Sample: Active recombinant IL8, Rabbit

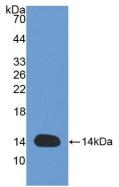


Figure 5. Western Blot

Sample: Recombinant IL8, Rabbit;

Antibody: Rabbit Anti-Rabbit IL8 Ab (PAA080Rb51)