APA860Hu02 100µg Active Epithelial Neutrophil Activating Peptide 78 (ENA78) Organism Species: *Homo sapiens (Human) 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: Arg45~Asn114 Tags: N-terminal His-tag Purity: >95% 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: 9.1 Predicted Molecular Mass: 11.8kDa Accurate Molecular Mass: 12kDa 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>]

RELRCV CLQTTQGVHP KMISNLQVFA IGPQCSKVEV VASLKNGKEI CLDPEAPFLK KVIQKILDGG NKEN

[ACTIVITY]

Epithelial Neutrophil Activating Peptide 78 (ENA-78) is a member of the CXC subfamily of chemokines. Full-length ENA-78 is 114 amino acids (aa) in length with a predicted molecular weight of 12 kDa. ENA-78 is expressed by many immune cells, such as macrophages, eosinophils, as well as non-immune cells including mesothelial cells, and fibroblasts. Soluble ENA-78 potently chemoattracts T cells and monocytes, while the cell-bound chemokine promotes strong adhesion of leukocytes to activated endothelial cells, where it is primarily expressed. Thus, chemotaxis assay used 24-well microchemotaxis system was undertaken to detect the chemotactic effect of ENA-78 on THP-1 the human monocytic cell line. Briefly, THP-1 cells were seeded into the upper chambers (150ul cell suspension, 10⁶ cells/ml in RPMI 1640 with FBS free) and different concentrations of ENA-78 diluted with serum free RPMI 1640 was added in lower chamber with a polycarbonate filter (8 um pore size) used to separate the two compartments. After incubation at 37 $^{\circ}$ C with 5% CO₂ 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 ENA-78 is able to induce migration of THP-1 cells. The migrated THP-1 cells in low chamber at low magnification(×100)

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were shown in Figure 1. Statistical results of were shown in Figure 2. The optimum chemotaxis of ENA-78 occurs at 1ug/ml.

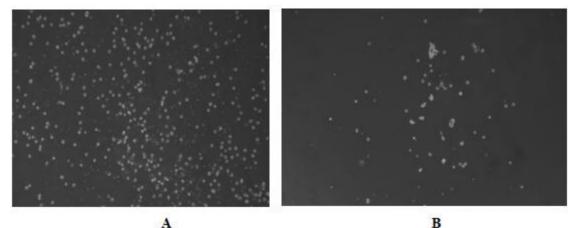
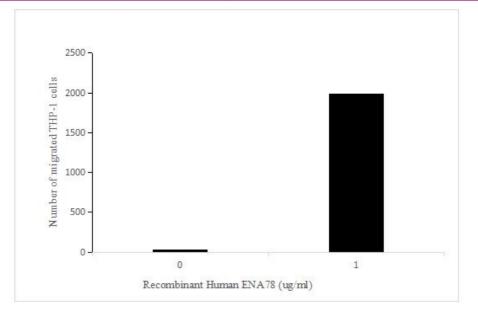


Figure 1. The chemotactic effect of ENA-78 on THP-1 cells

(A) THP-1 cells were seeded into the upper chambers and serum free RPMI 1640 with 1 ug/mI ENA-78 was added in lower chamber, then cells in lower chamber were observed at low magnification (×100) after incubation at 37 $^{\circ}$ C for 1h;

(B) THP-1 cells were seeded into the upper chambers and serum free RPMI 1640 without ENA-78 was added in lower chamber, then cells in lower chamber were observed at low magnification (×100) after incubation at 37 $\,^{\circ}$ C for 1h.

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[IDENTIFICATION]

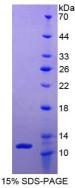


Figure 3. SDS-PAGE

Sample: Active recombinant ENA78, Human

[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.