

**APA860Hu03 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

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12th Edition (Revised in Aug, 2016)

## **[ PROPERTIES ]**

**Source:** Prokaryotic expression.

**Host:** *E. coli*

**Residues:** Ala41~Asn114

**Tags:** N-terminal His and GST Tag

**Purity:** >97%

**Traits:** Freeze-dried powder

**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:** 7.2

**Predicted Molecular Mass:** 38.1kDa

**Accurate Molecular Mass:** 40kDa as determined by SDS-PAGE reducing conditions.

## **[ USAGE ]**

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.

**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 ]**

```
AAVLRRLRCV  
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,  $10^6$  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 °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 ( $\times 10$ ) and

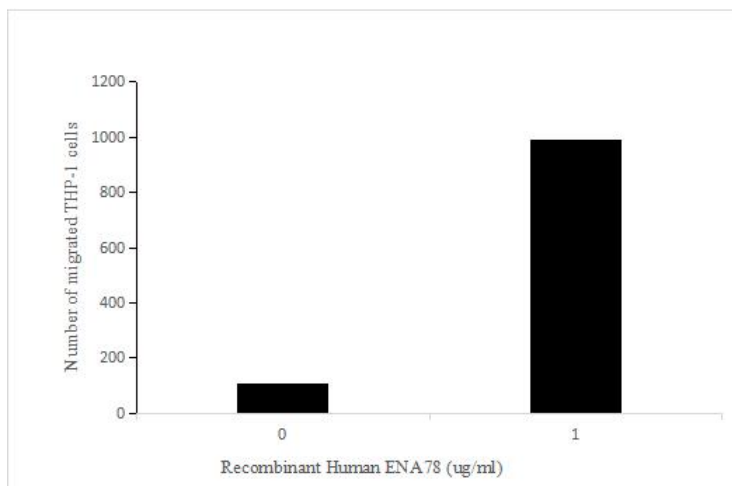
the number of migrated cells were counted using Fluorescence Activating Cell Sorter. Result shows ENA-78 is able to induce migration of THP-1 cells. The migrated THP-1 cells in low chamber at low magnification( $\times 10$ ) were shown in Figure 1. Statistical results of FACS were shown in Figure 2. The optimum chemotaxis of ENA-78 occurs at 1ug/ml.



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

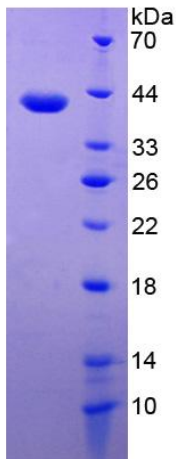
(A) Cells in lower chamber after incubation at 37 °C for 1h in which THP-1 cells were seeded into the upper chambers and 1 ug/ml ENA-78 was added in lower chamber.

(B) Cells in lower chamber after incubation at 37 °C for 1h in which THP-1 cells were seeded into the upper chambers and serum free RPMI 1640 without ENA-78 was added in lower chamber.



**Figure 2. The chemotactic effect of ENA-78 on THP-1 cells**

**[ IDENTIFICATION ]**



15% SDS-PAGE

**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.