

**APA482Ra01 10µg**  
**Active Endothelin 1 (EDN1)**  
**Organism Species: *Rattus norvegicus (Rat)***  
***Instruction manual***

FOR RESEARCH USE ONLY  
NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

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1st Edition (Apr, 2016)

## **[ PROPERTIES ]**

**Source:** Prokaryotic expression.

**Host:** *E. coli*

**Residues:** Ser54~His202

**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:** 9.7

**Predicted Molecular Mass:** 18.5kDa

**Accurate Molecular Mass:** 19kDa as determined by SDS-PAGE reducing conditions.

## **[ USAGE ]**

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.

**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

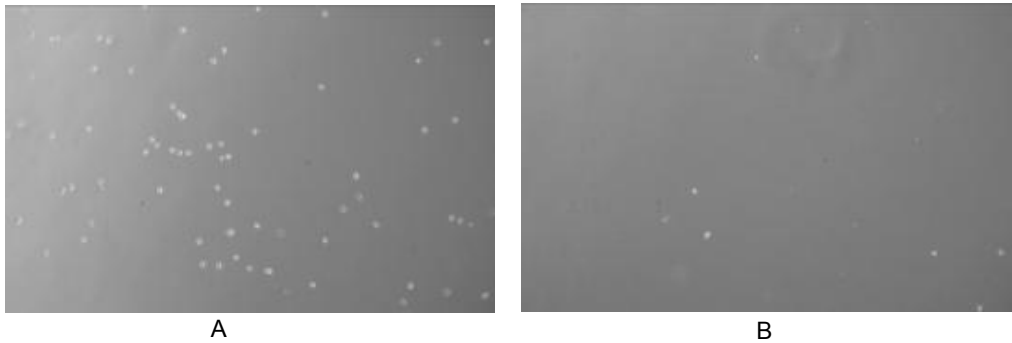
protein at 37°C for 48h, and no obvious degradation and precipitation were observed. The loss rate is less than 5% within the expiration date under appropriate storage condition.

## **[ SEQUENCE ]**

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SCSSLMD KECVYFCHLD IIWVNTPERV VPYGLGSPSR SKRSLKDLLP  
TKTTDQGNRC QCAHQKDKKC WNFQADKEL RAQSTMQKGV KDFKKGKPCP  
KLGKKCIYQQ LVEGRKLRLR EAISNSIKTS FRVAKLKAEL YRDQKLIHNR  
AH
```

## **[ ACTIVITY ]**

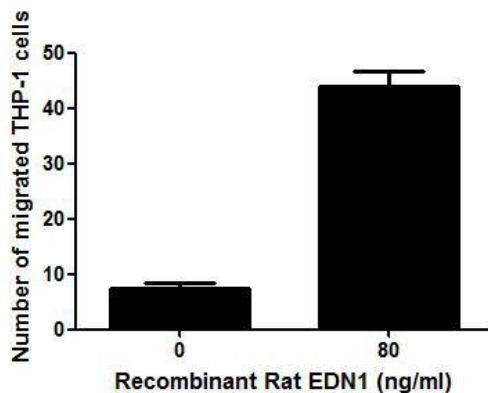
Endothelins (EDN) are small (21 amino acids) vasoactive peptides produced by many cell types including endothelial and epithelial cells, macrophages and fibroblasts. By binding to G-protein-linked transmembrane receptors, EDNs participate in vasoconstriction modulation and cell growth regulation. It has been proven that EDN1 has chemotaxis active on monocytes, thus chemotaxis assay used 24-well microchemotaxis system was undertaken to detect the chemotactic effect of EDN1 on the human monocytic cell line THP-1. Briefly, THP-1 cells were seeded into the upper chambers (100uL cell suspension, 106cells/mL in RPMI 1640 with 0.5%FBS) and EDN1 (20ng/mL, 40ng/mL and 80ng/mL diluted separately in serum free RPMI 1640) was added in lower chamber with a polycarbonate filter (8µm pore size) used to separate the two compartments. After incubation at 37°C with 5%CO<sub>2</sub> for 3h, 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 EDN1 is able to induce migration of THP-1 cells. The migrated THP-1 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 EDN1 occurs at 80ng/mL.



**Figure 1. The chemotactic effect of EDN1 on THP1 cells.**

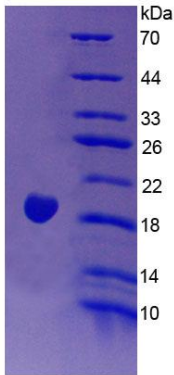
**(A)** THP-1 cells were seeded into the upper chambers and serum free RPMI 1640 with 80ng/mL EDN1 was added in lower chamber, then cells in lower chamber were observed at low magnification ( $\times 100$ ) after incubation for 3h;

**(B)** THP-1 cells were seeded into the upper chambers and serum free RPMI 1640 without EDN1 was added in lower chamber, then cells in lower chamber were observed at low magnification ( $\times 100$ ) after incubation for 3h.



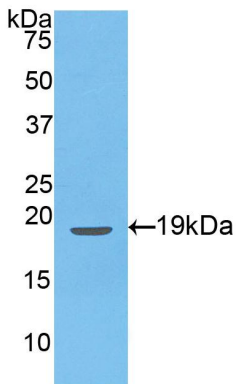
**Figure 2. The chemotactic effect of EDN1 on THP-1 cells.**

**[ IDENTIFICATION ]**



**Figure 3. SDS-PAGE**

**Sample: Active recombinant EDN1, Rat**



**Figure 4. Western Blot**

**Sample: Recombinant EDN1, Rat;**

**Antibody: Rabbit Anti- Rat EDN1 Ab (PAA482Ra01)**

**[ 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.