

**APA041Hu01 100µg**

**Active Active Neutrophil Activating Protein 3 (NAP3)**

**Organism Species: *Homo sapiens* (Human)**

***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:** Ala35~Asn107

**Tags:** Two N-terminal Tags, His-tag and GST-tag

**Purity:** >98%

**Endotoxin Level:** <1.0EU per 1µg (determined by the LAL method).

**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:** 8.3

**Predicted Molecular Mass:** 38.0kDa

**Accurate Molecular Mass:** 40kDa 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.

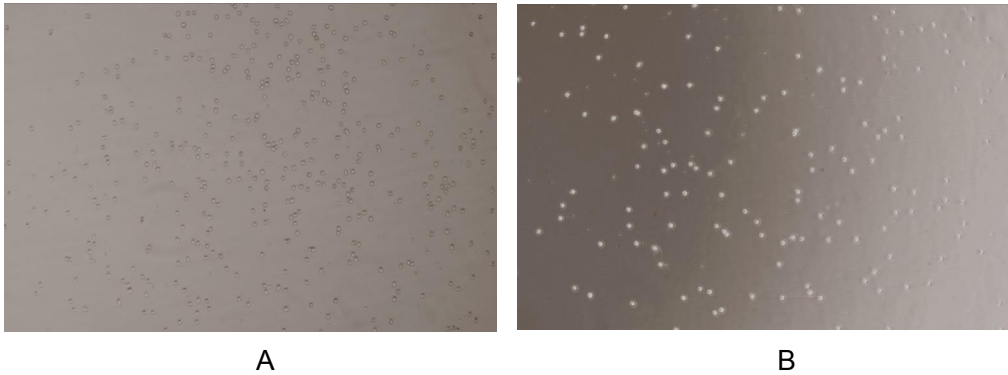
**Stability Test:** The thermal stability is described by the loss rate. The loss rate was determined by accelerated thermal degradation test, that is, incubate the protein at 37°C for 48h, and no obvious degradation and precipitation were observed. The loss rate is less than 5% within the expiration date under appropriate storage condition.

### **[ SEQUENCE ]**

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ASVATE LRCQCLQTLQ  
GIHPKNIQSV NVKSPGPHCA QTEVIATLKN GRKACLNPAS PIVKKIIEKM  
LNSDKSN
```

### **[ ACTIVITY ]**

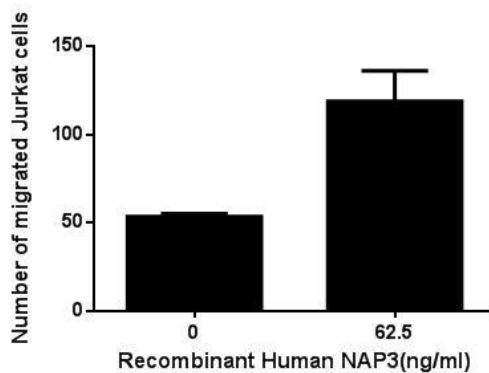
Neutrophil-activating protein 3 (NAP3) also known as chemokine (C-X-C motif) ligand 1 (CXCL1) is a small cytokine belonging to the CXC chemokine family. NAP3 is expressed by macrophages, neutrophils and epithelial cells, and has neutrophil chemoattractant activity. Thus, chemotaxis assay used 24-well microchemotaxis system was undertaken to detect the chemotactic effect of NAP3 on the human monocytic cell line THP-1. Briefly, THP-1 cells were seeded into the upper chambers (100uL cell suspension, 10<sup>6</sup> cells/mL in RPMI 1640 with FBS free) and SLC (31.25ng/mL, 62.5ng/mL, 125ng/mL and 250ng/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 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 NAP3 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 NAP3 occurs at 31.25-250ng/mL.



**Figure 1. The chemotactic effect of NAP3 on THP-1 cells.**

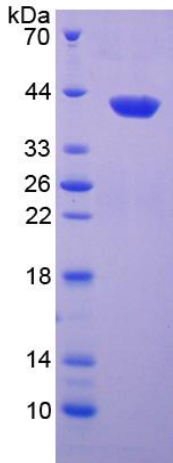
**(A)** THP-1 cells were seeded into the upper chambers and serum free RPMI 1640 with 62.5ng/mL NAP3 was added in lower chamber, then cells in lower chamber were observed at low magnification ( $\times 100$ ) after incubation for 1h;

**(B)** THP-1 cells were seeded into the upper chambers and serum free RPMI 1640 without NAP3 was added in lower chamber, then cells in lower chamber were observed at low magnification ( $\times 100$ ) after incubation for 1h.



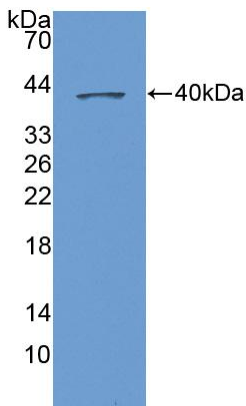
**Figure 2. The chemotactic effect of NAP3 on THP-1 cells.**

**[ IDENTIFICATION ]**



**Figure 2. SDS-PAGE**

**Sample: Active recombinant NAP3, Human**



**Figure 3. Western Blot**

**Sample: Recombinant NAP3, Human;**

**Antibody: Rabbit Anti-Human NAP3 Ab (PAA041Hu01)**

**[ IMPORTANT NOTE ]**

The kit is designed for in vitro and research use only, we will not be responsible for any issue if the kit was used in clinical diagnostic or any other procedures.