

**APA899Ra61 50µg**

**Active Osteopontin (OPN)**

**Organism Species: *Rattus norvegicus* (Rat)**

***Instruction manual***

FOR RESEARCH USE ONLY

NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

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13th Edition (Revised in Aug, 2023)

## **[ PROPERTIES ]**

**Source:** Eukaryotic expression.

**Host:** 293F cell

**Residues:** Leu17~Asn317

**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% sucrose.

**Applications:** Cell culture; Activity Assays; In vivo assays.

(May be suitable for use in other assays to be determined by the end user.)

**Predicted isoelectric point:** 4.3

**Predicted Molecular Mass:** 34.9kDa

**Accurate Molecular Mass:** 60kDa as determined by SDS-PAGE reducing conditions.

**Phenomenon explanation:**

The possible reasons that the actual band size differs from the predicted are as follows:

1. Splice variants: Alternative splicing may create different sized proteins from the same gene.
2. Relative charge: The composition of amino acids may affects the charge of the protein.
3. Post-translational modification: Phosphorylation, glycosylation, methylation etc.
4. Post-translation cleavage: Many proteins are synthesized as pro-proteins, and then cleaved to give the active form.
5. Polymerization of the target protein: Dimerization, multimerization etc.

## **[ USAGE ]**

Reconstitute in 10mM PBS (pH7.4) to a concentration of 0.1-1.0 mg/mL. Do not



## [ 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 ]

```
LPVK VAEFGSSEEK AHYSKHSDAV ATWLKPDPSQ
KQNLLAPQNS VSSEETDDFK QETLPSNSNE SHDHMDDDDD DDDGDHAES
EDSVNSDESD ESHHSDESDE SFTASTQADV LTPIAPTVDV PDGRGDSLAY
GLRSKRSRFP VSDEQYPDAT DEDLTSRMKS QESDEAIKVI PVAQRLSVPS
DQDSNGKTSH ESSQLDEPSV ETHSLEQSKE YKQRASHEST EQSDAIDSAE
KPAIDSAER SDAIDSQASS KASLEHQ SHE FHSHEDKLVL DPKSKEDDRY
LKFRISHELE SSSSEVN
```

## [ ACTIVITY ]

Osteopontin (OPN), a multifunctional phosphorylated glycoprotein, plays an important role in neutrophil recruitment and was found to induce the expression of proinflammatory chemokines including MCP-1 and MIP-1 $\beta$  which promote migration and recruitment of inflammatory cells. It has been reported that OPN induces MCP-1 expression through the NF-kappaB pathways in MCF-7 breast cancer cell line. To test the bioactivity of recombinant rat OPN, MCF-7 cells were seeded into 24-well plate at a density of  $1 \times 10^5$  cells/mL, and allowed to attach overnight before treated with certain concentrations of recombinant rat OPN for 48h and MCP-1 levels in the cell supernatant were determined by ELISA (SEA087Hu). MCP-1 levels in the cell supernatant of MCF-7 cells increased significantly after stimulated with OPN which was shown in Figure1, the EC50 was 9.83 ug/ml.

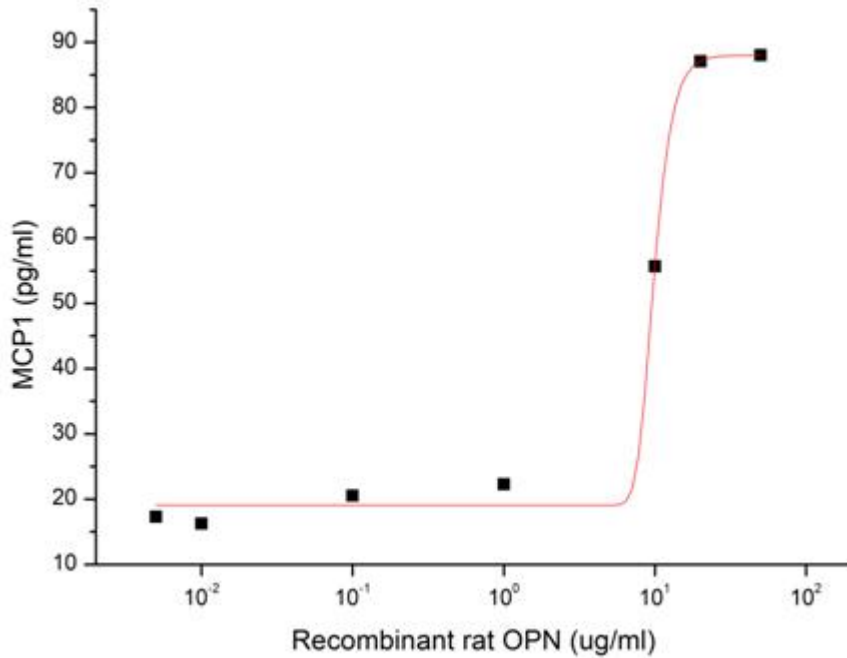


Figure1. MCP-1 levels in the cell supernatant of MCF-7 cells induced by OPN

[ IDENTIFICATION ]

CTCCGGTGAMGTGGCTGAGTTTGGGAGCTGAGAGAGAGGGGCAATTTCAGCAACCTGAGATCTGTAGGCGCTTGGCTGAGGCTGACCGATCTCTGAGAGGCAATCTTAGGCGGACAGAAATTCGTGTCTCTGAGAGAGGAGTGCCTTAGGCAAGAACTCTTCGAGGCTCTCAATGAA  
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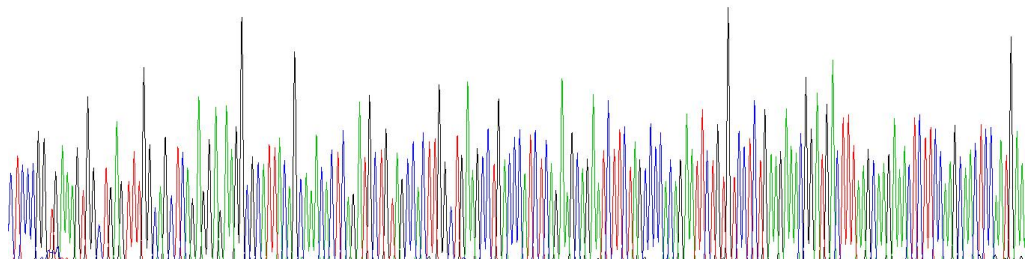
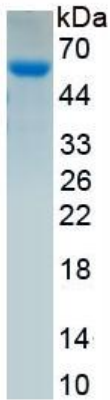


Figure 2. Gene Sequencing (extract)



**Figure 3. SDS-PAGE**

**Sample: Active recombinant OPN, Rat**

**[ 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.