APA899Ra61 100μg
Active Osteopontin (OPN)
Organism Species: Rattus norvegicus (Rat)
Instruction manual

FOR IN VITRO USE AND RESEARCH USE ONLY
NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

1th Edition (Apr, 2016)

[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: 20mM Tris, 150mM NaCl, pH8.0, containing 1mM EDTA, 1mM DTT, 0.01% sarcosyl, 5% trehalose, and Proclin300.

Predicted isoelectric point: 4.3

Predicted Molecular Mass: 34.9kDa

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

Applications: Cell culture; Activity Assays; In vivo assays.
(May be suitable for use in other assays to be determined by the end user.)

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 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 2-8ºC for one month.

Aliquot and store at -80ºC 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 37ºC 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 DDDDGDHAES
EDSVNSDESD ESHHSDESDE SFTASTQADV LTPIAPTVDV PDGRGDSLAY
GLRSKSRSFP VSDEQYPDAT DEDLTSRMKS QESDEAIKVI PVAQRLSVPS
DQDSNGKTSH ESSQLDEPSV ETHSLEQSKE YKQRASHEST EQSDAIDSAE
KPDAIDSAER SDAIDSQASS KASLEHQSHE 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-1β which promote migration and recruitment of inflammatory cells. It has been reported that OPN induces MCP-1 expression through the NF-kappa B pathways in MCF-7 breast cancer cell line. Briefly, MCF-7 cells were seeded overnight at a density of $1x10^5$ cells/mL, and treated with or without various concentrations of OPN for 24h and

MCP-1 levels in the cell supernatant were determined by ELISA.

MCP-1 levels in the cell supernatant of MCF-7 cells increased significantly after stimulated with OPN, the data was shown in Table 1 and Figure 1.

Sample (cell supernatant of MCF-7 cells)	O.D. value	Corrected	Concentration of MCP-1 (ng/mL)
stimulated with OPN (100ng/mL)	1.358	1.305	2.53
stimulated with OPN (200ng/mL)	1.177	1.124	2.15
stimulated with OPN (400ng/mL)	1.101	1.048	1.99
unstimulated	0.944	0.891	1.66

Table 1. MCP-1 levels in the cell supernatant of MCF-7 cells regulated by OPN.

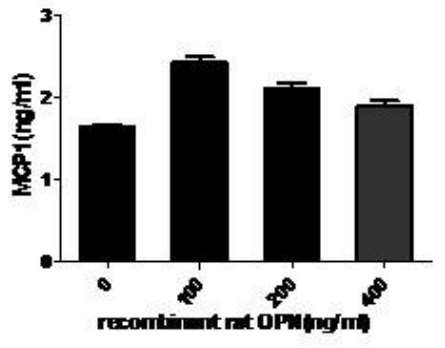

Figure 1. MCP-1 levels in the cell supernatant of MCF-7 cells regulated by OPN.

[IDENTIFICATION]

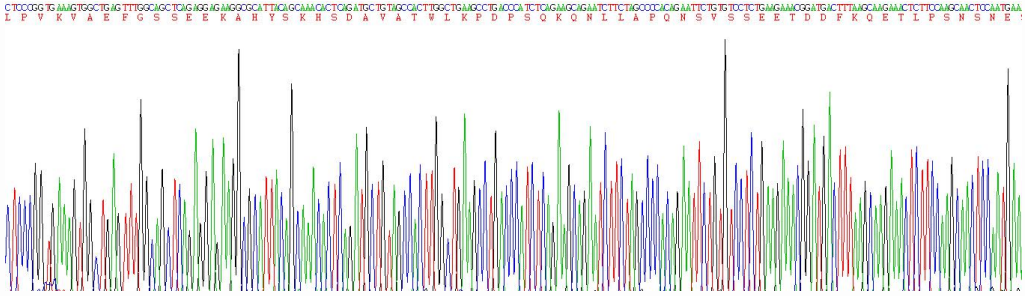

Figure 2. Gene Sequencing (extract)

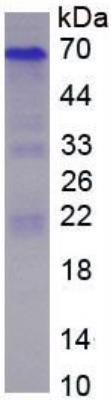

Figure 3. SDS-PAGE

Sample: Active recombinant OPN, Rat

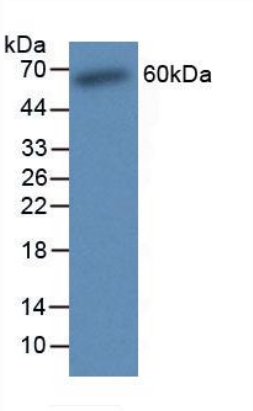

Figure 4. Western Blot

Sample: Recombinant OPN, Rat;

Antibody: Rabbit Anti-Rat OPN Ab (PAA899Ra06)