APA899Hu61 100μg
Active Osteopontin (OPN)
Organism Species: Homo sapiens (Human)
Instruction manual

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

1th Edition (Apr, 2016)

[PROPERTIES]

Source: Prokaryotic expression.
Host: *E. coli*
Residues: Ile17~Asn287
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: 32.2kDa
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 2-8°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]

IPVK QADSGSSEEK QNAVSSEETN DFKQETLPSK
SNESHDHMDD MDDEDDDDHV DSQDSIDSND SDDVDDTDDS HQSDESHHSD
ESDELVTDFP TDLPATEVFT PVVPTVDTYD GRGDSVVYGL RSKSKKFRRP
DIQYPDATDE DITSHMESEE LNGAYKAIPV AQDLNAPSDW DSRGKDSYET
SQLDDQSAET HSHKQSRLYK RKANDESNEH SDVIDSQELS KVSREFHSHE
FHSHEDMLVV DPKSKEEDKH LKFRISHELD SASSEVN

[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 would 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 OPN(100ng/ml、 200ng/ml) 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)	0.970	0.922	1.72
stimulated with OPN (200ng/mL)	1.155	1.107	2.11
unstimulated	0.674	0.626	1.11

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

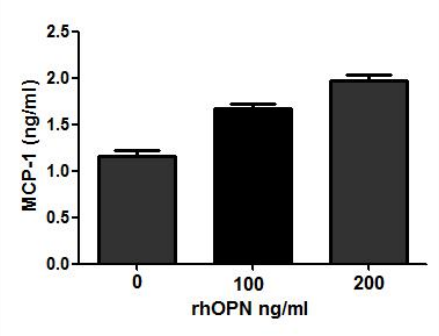

Figure 1. MCP-1 levels in the cell supernatant of MCF-7 cells regulated by OPN.

[IDENTIFICATION]

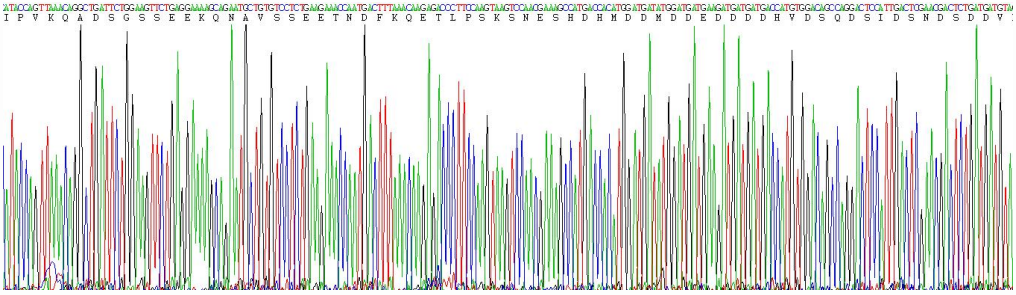

Figure 2. Gene Sequencing (extract)

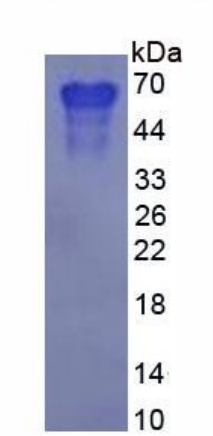

Figure 3. SDS-PAGE

Sample: Active recombinant OPN, Human

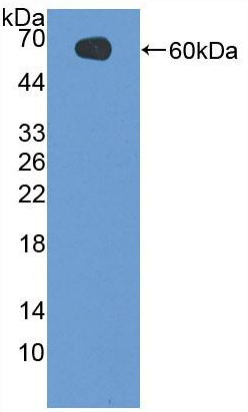

Figure 4. Western Blot

Sample: Recombinant OPN, Human;

Antibody: Rabbit Anti-Human OPN Ab (PAA899Hu06)