

**APA791Hu61 100µg  
Active Osteonectin (ON)**

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

***Instruction manual***

FOR RESEARCH USE ONLY

NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

---

---

13th Edition (Revised in Aug, 2023)

## **[ PROPERTIES ]**

**Source:** Eukaryotic expression.

**Host:** 293F cell

**Residues:** Ala18~Ile303

**Tags:** N-terminal His-tag

**Purity:** >95%

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

**Buffer Formulation:** PBS, pH7.4, containing 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:** 4.4

**Predicted Molecular Mass:** 34.3kDa

**Accurate Molecular Mass:** 44kDa 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 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 ]

```
APQ QEALPDETEV VEETVAEVTE VSVGANPVQV
EVGEFDDGAE ETEEEVVAEN PCQNHCKHG KVCELNNT PMCVCQDPTS
CPAPIGEFEK VCSNDNKTFD SSCHFFATKC TLEGTKKGHK LLDYIGPCK
YIPPCLDSEL TEFPLMRDW LKNVLVTLYE RDEDNNLLTE KQKLRVKKIH
ENEKRLEAGD HPVELLARDF EKNYNMYIFP VHWQFGQLDQ HPIDGYLSHT
ELAPLRAPLI PMEHCSTRFF ETCDLNDKY IALDEWAGCF GIKQKIDDKD
LVI
```

## [ ACTIVITY ]

SPARC, also known as osteonectin (ON), is the founding member of a family of secreted matricellular proteins with similar domain structure. The 302 amino acid (aa), 43 kDa protein contains a 17 aa signal sequence, an N-terminal acidic region that binds calcium, a follistatin domain containing Kazal-like sequences, and a C-terminal extracellular calcium (EC) binding domain with two EF-hand motifs. SPARC is produced by fibroblasts, capillary endothelial cells, platelets and macrophages, especially in areas of tissue morphogenesis and remodeling. SPARC shows context-specific effects, but generally inhibits adhesion, spreading and proliferation, and promotes collagen matrix formation. Besides, Collagen Type I Alpha 2 (COL1a2) has been identified as an interactor of SPARC, thus a functional binding ELISA assay was conducted to detect the interaction of recombinant human SPARC and recombinant human COL1a2. Briefly, SPARC was diluted serially in PBS with 0.01% BSA (pH 7.4). Duplicate samples of 100 µl were then transferred to COL1a2-coated microtiter wells and incubated for 1h at 37°C. Wells were washed with PBST and incubated for 1h

with anti-SPARC pAb, then aspirated and washed 3 times. After incubation with HRP labelled secondary antibody for 1h at 37°C, wells were aspirated and washed 5 times. With the addition of substrate solution, wells were incubated 15-25 minutes at 37°C. Finally, add 50 µL stop solution to the wells and read at 450/630 nm immediately. The binding activity of recombinant human SPARC and recombinant human COL1a2 was shown in Figure 1, and this effect was in a dose dependent manner.

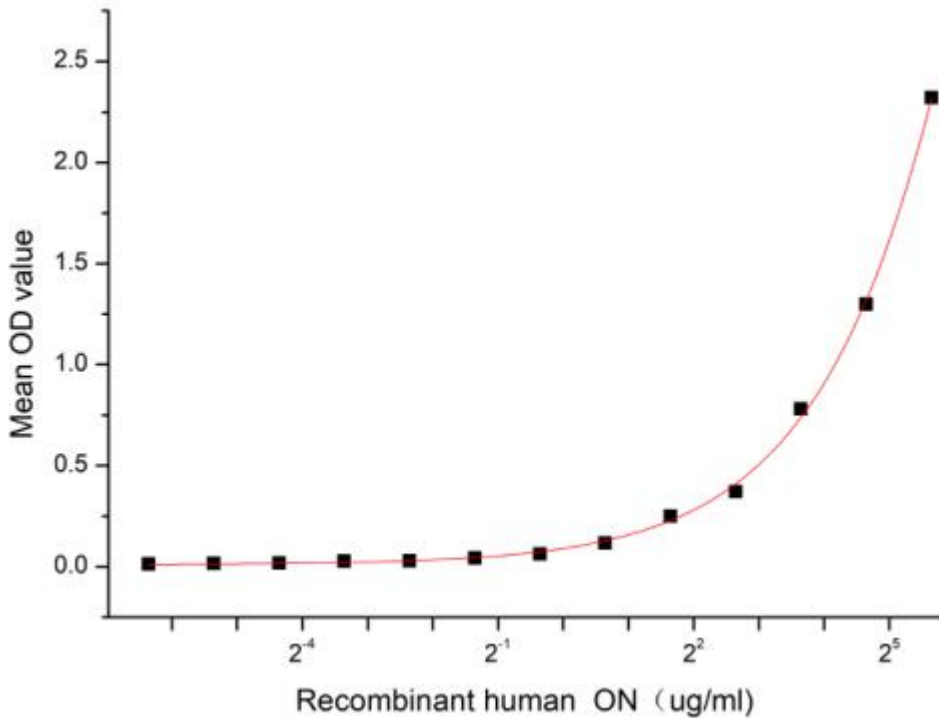
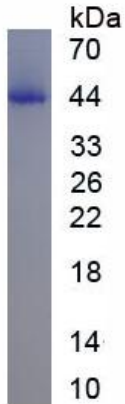


Figure 1. The binding activity of recombinant human ON and recombinant human COL1a2

**[ IDENTIFICATION ]**



**Figure 2. SDS-PAGE**

**Sample: Active recombinant ON, Human**

**[ 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.