

**APA037Po01 100µg**

**Active Fibronectin (FN)**

**Organism Species: *Sus scrofa*; *Porcine* (Pig)**

***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:** Prokaryotic expression.

**Host:** *E. coli*

**Residues:** Pro49~Asp403

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

**Purity:** >80%

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

**Buffer Formulation:** PBS, pH7.4, containing 0.01% Sarcosyl, 5%Trehalose .

**Original Concentration:** 200µg/mL

**Applications:** Activity Assays.

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

**Predicted isoelectric point:** 6.8

**Predicted Molecular Mass:** 43.2kDa

**Accurate Molecular Mass:** 45&33kDa 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 ]**

```
PGCYDNGKHYYQINQQWERTYLGSALVCTCYGGSRGFNCESKPEPEETCFDKYTGNTRYVG  
DTYERPKDSMIWDCTCIGAGRGRISCTIANRCHEGGQSYKIGDTWRRPHETGGYMLECVC  
LGNGKGEWTCKPIAEKCFDHAAGGTSYVVGETWEKPYQGWMVMVDCTCLGEGSGRITCTS  
RNRCDNDQDTRTSYRIGDTWSKKDNRGNLLQCICTGNRGGEWK CERHTSLQTTSAGSGSFT  
DVRTAIYQPQPHPQPAPYGHCVTD SGVVYSVGMQWLKTQGNKQMLCTCLGNGVSCQETA  
VTQTYGGNSNGEPCVLPFTYNGRTFYSCCTTEGRQDGHLCSTTSNYESQDQKYSFCTD
```

## **[ ACTIVITY ]**

Fibronectin (FN), a large, modular glycoprotein encoded by the FN1 gene, is a key component of the extracellular matrix (ECM) and plasma. Synthesized primarily by fibroblasts, hepatocytes, and endothelial cells, it exists in two major forms: soluble plasma FN and insoluble cellular FN. FN mediates critical cellular processes via its integrin-binding RGD motif, including cell adhesion, migration, proliferation, and differentiation. It also participates in ECM assembly, wound healing, and angiogenesis by bridging cells to other ECM components like collagen, fibrin, and proteoglycans. Dysregulated FN expression or function is linked to pathological

conditions such as fibrosis, cancer metastasis, and cardiovascular diseases, making it a vital target for biomedical research. FN binds THBS1, a secreted glycoprotein, to modulate ECM stability and regulate cellular adhesion signaling pathways. Briefly, THBS1 was diluted serially in PBS with 0.01% BSA (pH 7.4). Duplicate samples of 100  $\mu$ L were then transferred to FN-coated microtiter wells and incubated for 1h at 37°C. Wells were washed with PBST and incubated for 1h with anti-THBS1 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  $\mu$ L stop solution to the wells and read at 450/630nm immediately. Measured by its binding ability in a functional ELISA. When Recombinant FN is immobilized at 2  $\mu$ g/mL (100  $\mu$ L/well), the concentration of THBS1 that produces 50% optimal binding response is found to be approximately 0.054  $\mu$ g/mL.

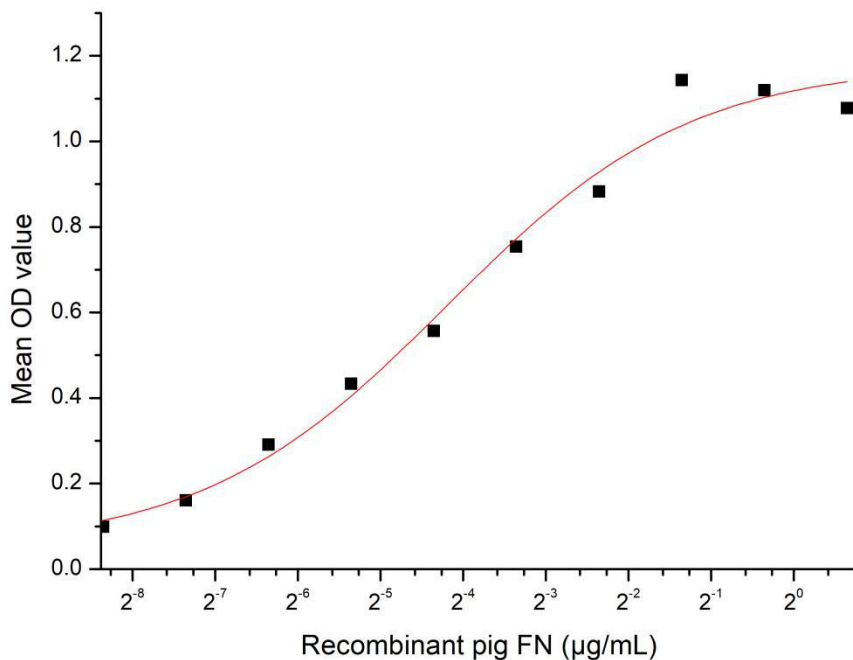


Figure 1. The binding activity of recombinant THBS1 and FN

## [ IDENTIFICATION ]

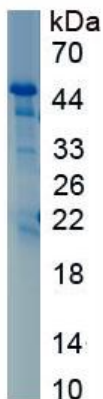


Figure 2. SDS-PAGE

Sample: Active recombinant FN, Pig

## [ 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.