

APB434Hu01 100µg

Active Proteinase 3 (PR3)

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: Prokaryotic expression.

Host: *E. coli*

Residues: His71~Leu247

Tags: N-terminal His-tag

Purity: >90%

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: 6.6

Predicted Molecular Mass: 20.8kDa

Accurate Molecular Mass: 21&17kDa 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]

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HCLRDIPQRL VNVVLGAHNV RTQEPTQQHF  
SVAQVFLNNY DAENKLNDVL LIQLSSPANL SASVATVQLP QDQPVPHGT  
QCLAMGWGRV GAHDPPAQVL QELNVTVVTF FCRPHNICTF VPRRKAGICF  
GDSGGPLICD GIIQGIDSFV IWGCATRLFP DFFTRVALYV DWIRSTL
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[ACTIVITY]

Proteinase 3 (PR3) is a serine protease mainly expressed in azurophilic granules of neutrophils. Structurally, it consists of a catalytic domain and a pro - domain. Functionally, PR3 is crucial for neutrophil - mediated immune responses. It can cleave various extracellular matrix proteins and bioactive peptides, playing roles in processes like neutrophil extracellular trap (NET) formation and degradation of pathogens. In diseases, abnormal PR3 activity is associated with autoimmune disorders such as granulomatosis with polyangiitis, where autoantibodies against PR3 are characteristic. The binding of PR3 to IL32 has been reported to enhance the inflammatory response. Thus a functional ELISA assay was conducted to detect the interaction of recombinant human PR3 and recombinant human IL32. Briefly, PR3 was diluted serially in PBS with 0.01% BSA (pH 7.4). Duplicate

samples of 100 μ l were then transferred to IL32-coated microtiter wells and incubated for 1h at 37°C. Wells were washed with PBST and incubated for 1h with anti-PR3 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 μ L stop solution to the wells and read at 450/630nm immediately. The binding activity of recombinant human PR3 and recombinant human IL32 was shown in Figure 1, the EC₅₀ for this effect is 0.026 μ g/mL.

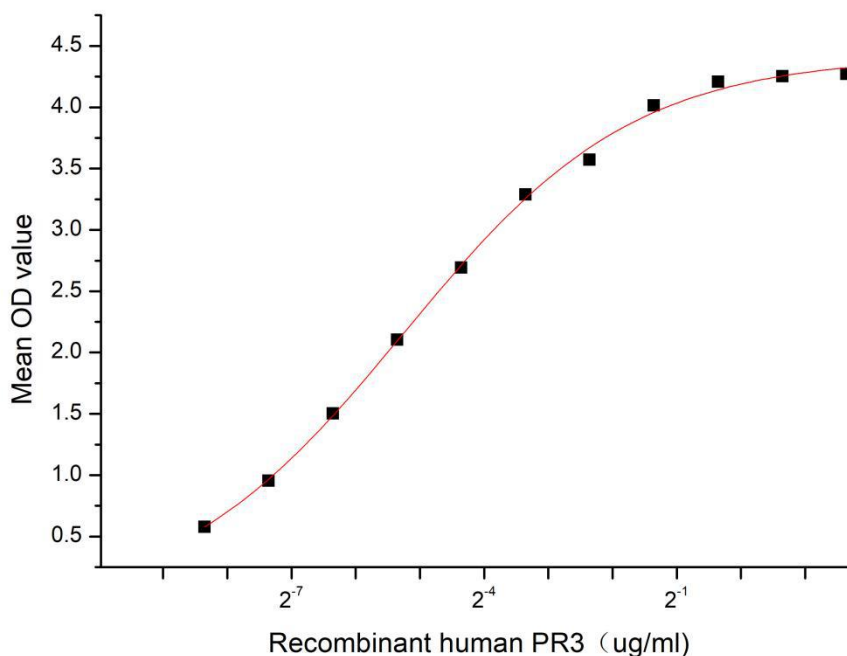


Figure 1. The binding activity of recombinant human PR3 and human IL32

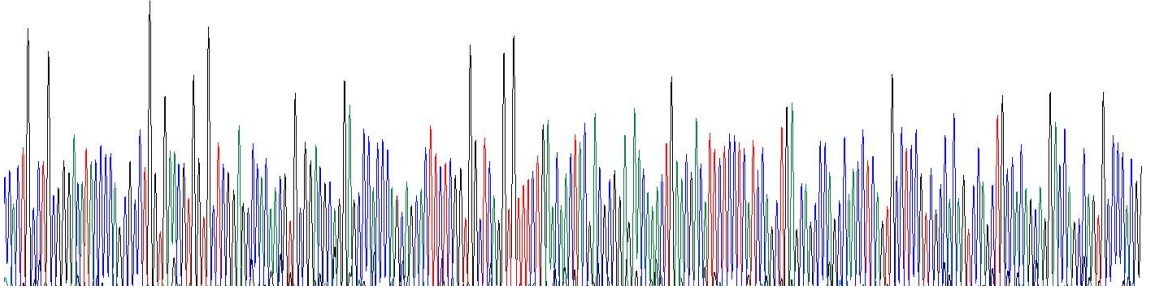
[illegible]

Figure 2. Gene Sequencing (extract)

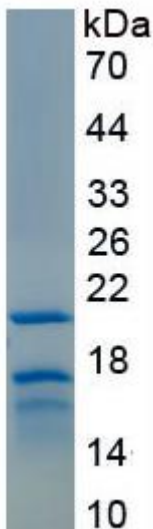


Figure 3. SDS-PAGE

Sample: Active recombinant PR3, 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.