

APA968Po61 100µg

Active Aprotinin (AP)

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

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: Pro2~Gly56

Tags: N-terminal His-tag

Purity: >90%

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

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

Original Concentration: 200µg/mL

Applications: Cell culture; Activity Assays.

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

Predicted isoelectric point: 6.3

Predicted Molecular Mass: 8.0kDa

Accurate Molecular Mass: 11kDa as determined by SDS-PAGE reducing conditions.

[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]

PDFCLEPPYTGPCCKARMIKYFYNI~~RS~~SCEEF~~I~~YGGCEAKKNNFEAMEDCMRTCG

[ACTIVITY]

Aprotinin (AP) is a competitive serine protease inhibitor. Reversibly binds to and blocks the enzymatic active site. Inhibits a range of serine proteases including trypsin, chymotrypsin, kallikrein and plasmin. Inhibits cytopathogenic effect of SARS-CoV-2 and double-stranded RNA formation in SARS-CoV-2-infected cells. The activity of recombinant pig AP was measured by its ability to inhibit trypsin cleavage of a peptide substrate BAPNA in the assay buffer 200 mM Triethanolamine hydrochloride, 20 mM CaCl₂, pH 7.8. The reaction was performed in adding 20 μl 4 mg/mL trypsin diluted by 1mM HCl to 160 μl assay buffer and 20 ul 0.85% (w/v) NaCl and start the reaction by adding 100 μl of 1mg/ml BAPNA. Include a substrate blank containing 160 μl assay buffer, 20 μl 1mM HCl, 20 ul 0.85% (w/v) NaCl and 100 μL of 1mg/ml substrate. Rapidly mixing at 25 °C, then read at 405 nm in kinetic mode for 5 minutes using a microplate reader controlling the $\Delta A_{405nm}/min=0.08-0.12$. The 20 ul different concentrations of recombinant pig AP was incubated with 20 ul 4 mg/mL trypsin in 160 ul assay buffer at 25 °C for 10 minutes followed by adding 100 ul substrate, then read at 405 nm in kinetic mode for 5 minutes using a microplate reader. Under these conditions, the enzyme amount of 50% inhibition of trypsin activity per minute is defined as a unit. The specific activity of recombinant pig AP is >9000 U/mg.

Calculation

$$\text{AP activity (U/mg)} = \frac{\frac{0.10 - A_{405}/\text{min}}{0.10} \times 100\%}{50\%} / M$$

Where:

- 0.10 = trypsin activity of $\Delta A_{405}/\text{min}$
- A_{405}/min = inhibition of trypsin activity of AP
- M = mass of enzyme

[IDENTIFICATION]

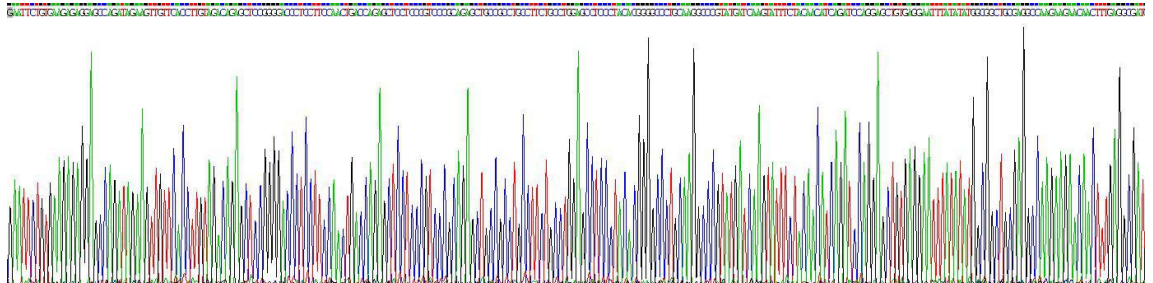


Figure 1. Gene Sequencing (extract)

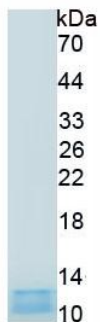


Figure 2. SDS-PAGE

Sample: Active recombinant AP, 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.