APA968Bo63 100μg
Active Aprotinin (AP)
Organism Species: *Bos taurus; Bovine (Cattle)*
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: Arg36~Ala93
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: 9.1
Predicted Molecular Mass: 8.7kDa
Accurate Molecular Mass: 13kDa 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]

RPDFCLEPPY TGPCKARIIR YFYNAKAGLC QTFVYGGCRA KRNNFKSAED CMRTCGGA

[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 bovine 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 ℃, then read at 405 nm in kinetic mode for 5 minutes using a microplate

reader controlling the ΔA405nm/min=0.08-0.12. The 20 ul different concentrations of recombinant bovine AP was incubated with 20 ul 4 mg/mL trypsin in 160 ul assay buffer at 25℃ 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 bovine AP is >1500 U/mg.

Calculation

$$\text{AP activity (U/mg)} = \frac{\frac{0.10 - A405/\text{min}}{0.10} \times 100\%}{50\%} / M$$

Where:

0.10 = trypsin activity of ΔA405nm/min
A405/min= inhibition of trpsin activity of AP
M=mass of enzyme

[IDENTIFICATION]

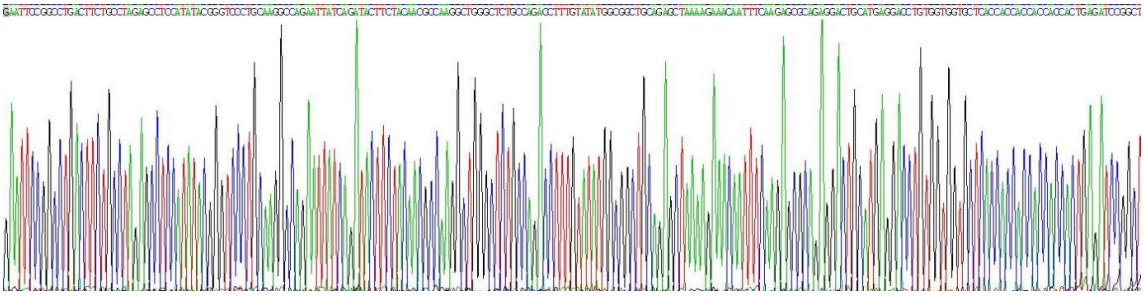

Figure 2. Gene Sequencing (extract)

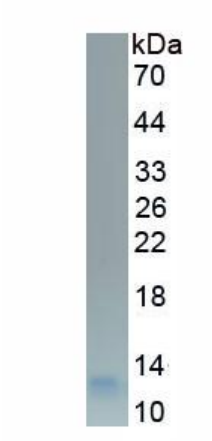

Figure 3. SDS-PAGE

Sample: Active recombinant AP, Cattle

[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.