APA968Bo62 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: >95%
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: 8.1kDa
Accurate Molecular Mass: 10kDa 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 2-8ºC for one month.
Aliquot and store at -80ºC 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 37ºC 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 >3000 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]

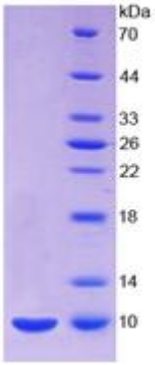

Figure 1. SDS-PAGE

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.