

APA476Po01 100µg

Active Cystatin A (CSTA)

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: Prokaryotic expression.

Host: *E. coli*

Residues: Met1~Phe103

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% SKL, 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: 5.8

Predicted Molecular Mass: 15.3kDa

Accurate Molecular Mass: 15kDa 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]

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MESDKMMPGG LTEARPATPE IQEIATTVKS QLEEKTNKTY EKFEAVEYKS  
QVVAGINYYI KVHVGGNSYV HIKVFKSLPY QNKPLELSGY QVDKTKDDEL  
TGF
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[ACTIVITY]

Cystatin A (CSTA) is a member of family 1 of the cystatin superfamily. Like Cystatin B, it is an intracellular inhibitor regulating the activities of cysteine proteases of the papain family such as cathepsins B, H and L. For example, immunohistochemical analysis of Cystatin A and cathepsin L is a useful indicator for malignancy in human epidermal keratinocytes. The ratio of cathepsin B and Cystatin A can be used in the differential diagnosis and treatment of patients with prostate carcinoma.

The activity of recombinant pig Cystatin A was measured by its ability to inhibit papain cleavage of a fluorogenic peptide substrate Z-FR-AMC in the assay buffer 50 mM Tris, pH 7.0. Papain was diluted to 500 ug/ml in activation buffer 50 mM Tris, 5 mM DTT, pH 7.0 and incubated at room temperature for 15 minutes. The activated papain was diluted to 100 ug/ml in the assay buffer and 20 ul different concentrations of recombinant pig Cystatin A (MW: 40.9 KD) was incubated with 20 ul 100 ug/ml papain at 37 °C for 10 minutes. Loading 50 µL of the incubated mixtures which were diluted five-fold in assay buffer into empty wells of a plate, and start the reaction by adding 50 µL of 200 µM substrate. Include a substrate blank containing 50 µL of assay buffer and 50 µL of 200 µM substrate. Then read at excitation and emission wavelengths of 380 nm and 460 nm, respectively, in kinetic mode for 5 minutes. The result was shown in Figure 1 and it was obvious that recombinant pig Cystatin A significantly decreased papain activity. The

inhibition IC₅₀ was <300 nM.

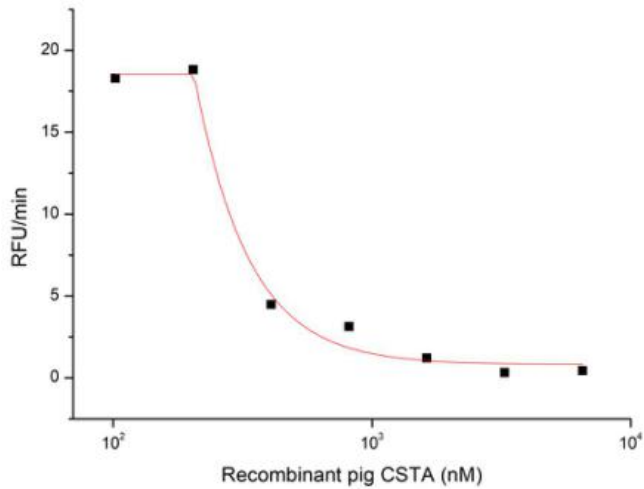


Figure 1. Inhibition of papain activity by recombinant pig CSTA

[IDENTIFICATION]

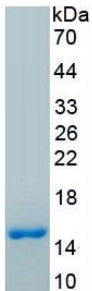


Figure 2. SDS-PAGE

Sample: Active recombinant CSTA, 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.