

**APJ325Hu01 100µg
Active Cystatin 5 (CST5)**

**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: Gly21~Val142

Tags: N-terminal His-tag

Purity: >95%

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: 8.3

Predicted Molecular Mass: 17.5kDa

Accurate Molecular Mass: 18kDa 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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GSASAQSRTL AGGIHATDLN DKSVCALDF AISEYNKVIN  
KDEYYSRPLQ VMAAYQQIVG GVNYYFNVKF GRTTCTKSQP NLDNCPFNDQ PKLKEEEFCS  
FQINEVPWED KISILNYKCR KV
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[ACTIVITY]

Cystatin D is a member of family 2 of the cystatin superfamily. In contrast to other members of family 2, Cystatin D has restricted tissue distribution and has been found only in saliva and tears. Two allelic variants (Arg46 and Cys46) are known in the human protein and they are not significantly different in their inhibitory activity against papain and cathepsins B, H, L and S. Recombinant Human Cystatin D corresponds to the Arg46 variant. The functions of Cystatin D are largely unknown. However, Cystatin D has been shown to inhibit coronavirus replication at its physiological concentration (0.12-1.9 μ M) and has been suggested to play a protective role against proteases present in the oral cavity. The activity of recombinant human Cystatin D 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 human Cystatin D (MW: 17.45 KD) was incubated with 20 ul 100 ug/ml papain at 37 °C for 10 minutes. Loading 50 μ L 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 μ L of 200 μ M substrate. Include a substrate blank containing 50 μ L of assay buffer and 50 μ L of 200 μ M 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 human Cystatin D significantly decreased papain activity. The inhibition IC₅₀ was <200 nM.

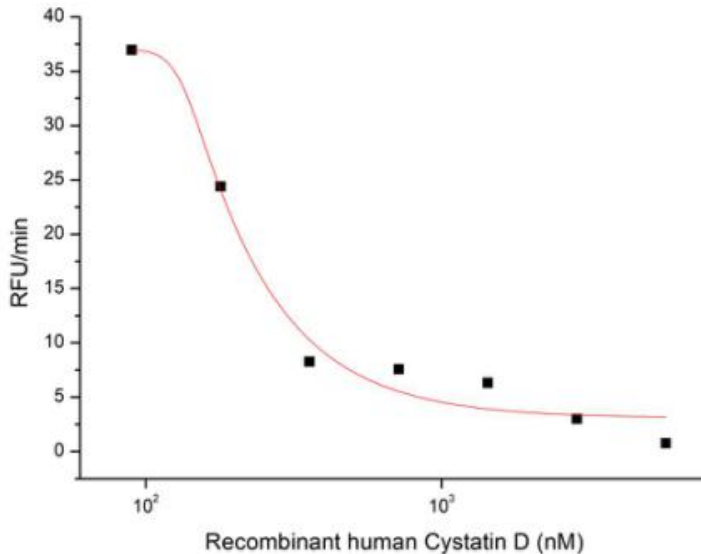


Figure 1. Inhibition of papain activity by recombinant human Cystatin D

[IDENTIFICATION]

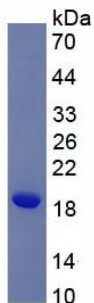


Figure 2. SDS-PAGE

Sample: Active recombinant CST5, 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.