Recombinant Human Cystatin D

Catalog Number: 1202-PI

DESCRIPTION

Source	Mouse myeloma cell line, NS0-derived Thr29-Val142, with a C-terminal 10-His tag Accession # CAA49838
N-terminal Sequence Analysis	Thr29
Predicted Molecular Mass	15 kDa

SPECIFICATIONS

SDS-PAGE	15 kDa, reducing conditions
Activity	Measured by its ability to inhibit papain cleavage of a fluorogenic peptide substrate Z-FR-AMC (Catalog # ES009). The IC_{50} value is <8 nM, under the described conditions. See Activity Assay Protocol on www.RnDSystems.com
Endotoxin Level	<1.0 EU per 1 µg of the protein by the LAL method.
Purity	>95%, by SDS-PAGE under reducing conditions and visualized by silver stain.
Formulation	Lyophilized from a 0.2 µm filtered solution in MES and NaCl. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Activation Buffer: 50 mM Tris, 5 mM DTT, pH 7.0
- Assay Buffer: 50 mM Tris, pH 7.0
- Recombinant Human Cystatin D (rhCystatin D) (Catalog # 1202-PI)
- Papain (Sigma, Catalog # P4762)
- Substrate: Z-Phe-Arg-AMC (Catalog # ES009), 10 mM stock in DMSO
- F16 Black Maxisorp Plate (Nunc, Catalog # 475515)
- Fluorescent Plate Reader (Model: SpectraMax Gemini EM by Molecular Devices) or equivalent

Assay

1. Chill Activation Buffer on ice.
2. Dilute Papain to 100 µg/mL in Activation Buffer.
3. Incubate at room temperature for 15 minutes.
4. Prepare a dilution curve of rhCystatin D (MW: 15,273 Da) in Assay Buffer. Make the following serial dilutions: 6000, 3000, 1000, 500, 250, 100, 50, 10, and 1 nM.
5. Dilute activated Papain to 2 µg/mL in Activation Buffer.
6. Mix equal volumes of the rhCystatin D curve dilutions and the diluted active Papain. Include a control (in duplicate) containing Assay Buffer and the diluted active Papain.
7. Incubate mixtures at 37 °C for 15 minutes.
8. Dilute Substrate to 200 µM in Assay Buffer.
9. Perform a five-fold dilution with Assay Buffer to the incubated mixture of rhCystatin D curve and Papain.
10. Load 50 µL of diluted incubated mixture into a plate, and start the reaction by adding 50 µL of 200 µM Substrate. Include a Substrate Blank by combining 50 µL of 200 µM Substrate and 50 µL Assay Buffer.
11. Read at excitation and emission wavelengths of 380 nm and 460 nm, respectively, for 5 minutes in kinetic mode.
12. Derive the 50% inhibition concentration (IC_{50}) for rhCystatin D by plotting RFU/min (or specific activity) vs. concentration with 4-PL fitting.
13. The specific activity for Papain at each point may be derived using the following formula (if needed):

$$\text{Specific Activity (pmol/min/µg)} = \frac{\text{Adjusted } V_{max}\text{* (RFU/min) x Conversion Factor** (pmol/RFU)}}{\text{amount of enzyme (µg)}}$$

*Adjusted for Substrate Blank

**Derived using calibration standard 7-Amino, 4-Methyl Coumarin (AMC) (Sigma, Catalog # A-9891).

Final Assay Conditions

Per Well:

- Papain: 0.010 µg
- Substrate: 100 µM
- rhCystatin D: 300, 150, 50, 25, 12.5, 5, 2.5, 0.5, and 0.05 nM

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 100 µg/mL in sterile 50 mM Tris, pH 7.0.
Shipping	The product is shipped with polar packs. Upon receipt, store it immediately at the temperature recommended below.
Stability & Storage	**Use a manual defrost freezer and avoid repeated freeze-thaw cycles.** ● 6 months from date of receipt, -20 to -70 °C as supplied. ● 3 months, -20 to -70 °C under sterile conditions after reconstitution.

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BACKGROUND

Cystatin D is a member of family 2 of the cystatin superfamily (1). 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 (2). 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 µM) and has been suggested to play a protective role against proteases present in the oral cavity (3).

References:

1. Freije, J.P. *et al.* (1993) J. Biol. Chem. **268**:15737.
2. Balbin, M. *et al.* (1994) J. Biol. Chem. **269**:23156.
3. Collins, A.R. and A. Grubb (1998) Oral Microbiol. Immunol. **13**:59.