Recombinant Human Cystatin E/M

Catalog Number: 1286-PI

DESCRIPTION

Source	Mouse myeloma cell line, NS0-derived Arg29-Met149, with a C-terminal 10-His tag Accession # Q15828
N-terminal Sequence Analysis	Arg29
Predicted Molecular Mass	15 kDa

SPECIFICATIONS

SDS-PAGE	15 kDa and 21 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 approximately 7.0 nM, as measured under the described conditions.
Endotoxin Level	<1.0 EU per 1 µg of the protein by the LAL method.
Purity	>95%, by SDS-PAGE visualized with Silver Staining and quantitative densitometry by Coomassie® Blue Staining.
Formulation	Lyophilized from a 0.2 µm filtered solution in MES and NaCl. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 50 mM Tris, pH 7.0
- Dithiothreitol (DTT) (Sigma, Catalog # D0632)
- Recombinant Human Cystatin E/M (rhCystatin E/M) (Catalog # 1286-PI)
- Papain (Sigma, Catalog # P-4762)
- 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 the Assay Buffer on ice.
2. Dilute Papain to 100 µg/mL in Assay Buffer with 5 mM DTT.
3. Incubate at room temperature for 15 minutes.
4. After incubation, dilute activated Papain to 2.05 ng/µL in Assay Buffer.
5. Prepare a curve of rhCystatin E/M (MW: 14,953Da) in Assay Buffer. Make the following serial dilutions: 2000, 500, 250, 125, 62.5, 31.25, 15.625, 5, and 1 nM.
6. Combine equal volumes of the rhCystatin E/M curve dilutions and 2.05 ng/µL active Papain. Include a control (in duplicate) containing equal volumes of Assay Buffer and 2.05 ng/µL Papain without adding any rhCystatin E/M.
7. Incubate mixtures at 37 °C for 10 minutes.
8. Dilute the reaction mixture 5 fold with Assay Buffer.
9. Dilute Substrate to 200 µM in Assay Buffer.
10. Load 50 µL of the incubated mixtures in a plate, and start the reaction by adding 50 µL of 200 µM Substrate.
    Read at excitation and emission wavelengths of 380 nm and 460 nm (top read), respectively in kinetic mode for 5 minutes.
11. Derive the 50% inhibiting concentration (IC_{50}) for rhCystatin E/M by plotting RFU/min (or specific activity) vs. concentration with 4-PL fitting.
12. The specific activity of Papain at each point may be determined 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 (Sigma, Catalog # A-9891).

Final Assay Conditions

Per Well:

- Papain: 0.01025 µg
- rhCystatin E/M curve: 100, 25, 12.5, 6.25, 3.125, 1.5625, 0.781, 0.25, and 0.05 nM
- Substrate: 100 µM

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 100 µg/mL in sterile 25 mM MES, 150 mM NaCl, pH 6.5.
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 E/M encoded by the CST6 gene is a member of family 2 of the cystatin superfamily (1, 2). It inhibits papain and cathepsin B, two of the cysteine proteases. Its mRNA was found in many tissues by the two groups who did initial cloning (1, 2). However, its protein was found only in skin and sweat glands by a third group (3). In addition to being a cysteine protease inhibitor, cystatin E/M is also a substrate for transglutaminases (3). It is required for viability and for correct formation of cornified layers in the epidermis and hair follicles, as ichq mice, with a null mutation in the cystatin E/M gene, have defects in epidermal cornification and die between 5 and 12 days of age (4). Cystatin E/M expression and function may not be limited to cutaneous epithelia. For example, it is found in rat brain and is induced during neuronal cell differentiation (5).

References:

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