Recombinant Human Chymase/CMA1

Catalog Number: 4099-SE

DESCRIPTION

Source	Mouse myeloma cell line, NS0-derived Met1-Asn247, with a C-terminal 10-His tag Accession # P23946
N-terminal Sequence Analysis	Gly20
Structure / Form	Pro form
Predicted Molecular Mass	27 kDa

SPECIFICATIONS

SDS-PAGE	38 kDa, reducing conditions
Activity	Measured by its ability to cleave the fluorogenic peptide substrate, SUC-Ala-Ala-Pro-Phe-AMC. The specific activity is >80 pmol/min/µg, as measured under the described conditions.
Endotoxin Level	<1.0 EU per 1 µg of the protein by the LAL method.
Purity	>90%, by SDS-PAGE under reducing conditions and visualized by silver stain.
Formulation	Supplied as a 0.2 µm filtered solution in HEPES and NaCl. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Maturation Buffer: 50 mM MES, pH 5.5
- Cathepsin Buffer: 50 mM MES, 50 mM NaCl, 5 mM DTT, pH 5.5
- Assay Buffer: 20 mM Tris, 2 M KCl, 0.02% (v/v) Triton® X-100, pH 9.0
- Recombinant Human Chymase/CMA1 (rhChymase) (Catalog # 4099-SE)
- Recombinant Mouse Active Cathepsin C/DPPI (rmCathepsin C) (Catalog # 2336-CY)
- Heparin (Sigma, Catalog # H3393), 20 mg/mL stock in deionized water
- N-Ethylmaleimide (NEM) (Sigma, Catalog # E1271), 50 mM stock in deionized water
- Substrate: SUC-Ala-Ala-Pro-Phe-AMC (Bachem, Catalog # I-1465), 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. Dilute rhChymase to 20 µg/mL in Maturation Buffer.
2. Dilute rmCathepsin C to 20 µg/mL in Cathepsin Buffer.
3. Dilute Heparin to 1.5 mg/mL in deionized water.
4. Combine 15 µL of 20 µg/mL rhChymase and 15 µL of 20 µg/mL rmCathepsin C.
5. Add 1 µL of Heparin and mix well.
6. Incubate at room temperature for 1 hour.
7. Stop maturation by adding 1.98 µL of 50 mM stock of NEM for a final concentration of 3 mM.
8. Dilute rhChymase to 2 µg/mL in Assay Buffer.
9. Incubate at room temperature for 5 minutes.
10. Dilute Substrate to 200 µM in Assay Buffer.
11. Load 50 µL of 2 µg/mL rhChymase in a plate, and start the reaction by adding 50 µL of 200 µM Substrate. As a Substrate Blank combine 50 µL of Substrate with 50 µL of Assay Buffer.
12. Read at excitation and emission wavelengths of 380 nm and 460 nm (top read), respectively, in kinetic mode for 5 minutes.
13. Calculate Specific Activity:

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

*Adjusted for Substrate Blank

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

Final Assay Conditions

Per Well:

- rhChymase: 0.100 µg
- Substrate: 100 µM

PREPARATION AND STORAGE

Shipping	The product is shipped with dry ice or equivalent. 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 opening.

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BACKGROUND

Chymases are a group of chymotrypsin-like serine proteases secreted by mast cells (1). They are synthesized as inactive precursors containing a 2-residue propeptide, which needs to be removed by dipeptidyl peptidase I/cathepsin C for the enzymatic activity (2). Human Chymase encoded by the CMA1 gene is known to be involved in hypertention and heart failure through its ability to convert angiotensin I (Ang I) to angiotensin II (Ang II), which plays a key role in the regulation of arterial pressure (3). In addition, it is also important in physiological and pathological conditions including inflammation, fibrosis and processing of cytokines (4). Therefore, designing a specific inhibitor for Chymase activity has been a pharmacologic strategy to develop therapeutic agents.

References:

1. Caughey, G.H. (2004) in *Handbook of Proteolytic Enzymes.* Barrett, A.J. *et al. ed.* p. 1531, Academic Press, San Diego.
2. Murakami, M. *et al.* (1995) J. Biol. Chem. **270**:2218.
3. Miyazaki, M. and S. Takai (2006) J. Pharmacol. Sci. **100**:391.
4. Nakajima, M. and N. Naya (2002) Jpn. J. Pharmacol. **90**:206.

PRODUCT SPECIFIC NOTICES

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