Recombinant Mouse Neutrophil Elastase/ELA2

Catalog Number: 4517-SE

DESCRIPTION

Source	Mouse myeloma cell line, NS0-derived Ser27-Arg260, with a C-terminal 10-His tag Accession # NP_056594
N-terminal Sequence Analysis	Ser27
Predicted Molecular Mass	27 kDa

SPECIFICATIONS

SDS-PAGE	34 kDa, reducing conditions
Activity	Measured by its ability to cleave the fluorogenic peptide substrate, MeOSuc-Ala-Ala-Pro-Val-7-amido-4-methylcoumarin (MeOSuc-AAPV-AMC). The specific activity is >500 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	>95%, by SDS-PAGE under reducing conditions and visualized by silver stain.
Formulation	Supplied as a 0.2 µm filtered solution in Tris and NaCl. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Activation Buffer: 50 mM MES, 50 mM NaCl, pH 5.5
- Assay Buffer: 50 mM Tris, 1 M NaCl, 0.05% (w/v) Brij-35, pH 7.5
- Recombinant Mouse Neutrophil Elastase/ELA2 (rmELA2) (Catalog # 4517-SE)
- Recombinant Mouse Active Cathepsin C/DPPI (rmCathepsin C) (Catalog # 2336-CY)
- Substrate: MeOSuc-Ala-Ala-Pro-Val-AMC (Bachem, Catalog # I-1270), 10 mM in DMSO
- F16 Black Maxisorp Plate (Nunc, Catalog # 475515)
- Fluorescent Plate Reader (Model: SpectraMax Gemini EM by Molecular Devices) or equivalent

Assay

1. Dilute rmELA2 to 50 µg/mL in Activation Buffer containing 50 µg/mL rmCathepsin C.
2. Incubate for 2 hours at 37 °C to activate rmELA2.
3. Dilute active rmELA2 to 1 ng/µL in Assay Buffer.
4. Dilute Substrate to 200 µM in Assay Buffer.
5. Load into a plate 50 µL of 1 ng/µL rmELA2, and start the reaction by adding 50 µL of 200 µM Substrate. Include a Substrate Blank containing 50 µL Assay Buffer and 50 µL of 200 µM Substrate.
6. Read at excitation and emission wavelengths of 380 nm and 460 nm (top read), respectively, in kinetic mode for 5 minutes.
7. 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:

- rmELA2: 0.05 µg
- Substrate: 100 µM

PREPARATION AND STORAGE

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 opening.

BACKGROUND

Neutrophil Elastase (ELA2), also known as polymorphonuclear leukocyte elastase, is a serine protease belonging to the chymotrypsin family. Located primarily in the azurophil granules of polymorphonuclear leukocytes, ELA2 function is the degradation of many extracellular matrix macromolecules (1, 2). α-1 Antitrypsin (Serpin A1) and secretory leukocyte protease inhibitor (SLPI) have been shown to inhibit ELA2 activity (3). This protein may be involved in lung emphysema, cystic fibrosis, the adult respiratory distress syndrome (ARDS), rheumatoid arthritis, tumor invasion and infectious diseases (1). The purified rmELA2 does not contain the last five amino acid residues of the deduced amino acid sequence (NP_056594).

References:

1. Bieth, J.G. (2004) In Handbook of Proteolytic Enzymes. Barrett, A.J., Rawlings, N.D., & Woessner, J.F. eds. pp. 1517.
2. Owen, C.A. *et al.* (1995) J. Cell Biol. **131**:775.
3. Wiesner, O. *et al.* (2005) FEBS letters **579**:5305.

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