**DESCRIPTION**

**Source**
Chinese Hamster Ovary cell line, CHO-derived Ser28-Asn252, with a C-terminal 10-His tag  
Accession # P08246

**N-terminal Sequence Analysis**
Ser28

**Predicted Molecular Mass**
25 kDa

**SPECIFICATIONS**

**SDS-PAGE**
29-39 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 >1,500 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 visualized with Silver Staining and quantitative densitometry by Coomassie® Blue Staining.

**Formulation**
Supplied as a 0.2 μm filtered solution in HEPES 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 Human Elastase/ELA2 (rhELA2) (Catalog # 9167-SE)
- Recombinant Mouse Active Cathepsin C/DPPI (rmCathepsin C) (Catalog # 2336-CY)
- Substrate: MEOSUC-Ala-Ala-Pro-Val-AMC (Bachem, Catalog # I1270), 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 rhELA2 to 50 μg/mL in Activation Buffer containing 50 μg/mL rmCathepsin C.
2. Incubate for 2 hours at 37 °C to activate rhELA2.
3. Dilute active rhELA2 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 rhELA2, 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:

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

*Adjusted for Substrate Blank.
**Derived using calibration standard 7-amino, 4-Methyl Coumarin (Sigma, Catalog # A9891).

**Final Assay Conditions**
Per Well:
- rhELA2: 0.05 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.
Neutrophil Elastase (ELA2, ELANE), also known as HNE, is a chymotrypsin family serine protease that plays a key role in pathogen clearance (1-3). It is expressed by promyelocytes and stored in the intracellular azurophilic granules of polymorphonuclear leukocytes (PMN) (4). These granules fuse with phagosomes, enabling Neutrophil Elastase to participate in the digestion and killing of endocytosed microbes. The enzyme is released by activated neutrophils at sites of inflammation, and it can remain associated with the cell surface or function as a component of neutrophil extracellular nets (NETs) which trap and kill microbial pathogens (5, 6). It also can degrade multiple extracellular matrix proteins including Elastin and Fibronectin (5). In the lung, this activity contributes to pathology in emphysema, cystic fibrosis, and adult respiratory distress syndrome (ARDS) (1). Neutrophil Elastase can be inhibited by Serpin A1/alpha 1-Antitrypsin, SLPI, Serpin B1, and Trappin-2/Elafin (7-11). Its activity in the lung is increased by exposure to tobacco smoke which inactivates Serpin A1 through methionine oxidation (12). Mature human Neutrophil Elastase shares 73% amino acid sequence identity with mouse and rat Neutrophil Elastase (13, 14). Multiple mutations in the human ELANE gene are causative of severe congenital and cyclic neutropenias (15).

References: