

DESCRIPTION

Source *E. coli*-derived
Ile24-Asn246, with an N-terminal Met, 6-His tag and GGSGSGGGSSR linker
Accession # NP_035775

N-terminal Sequence Analysis Ile24

Predicted Molecular Mass 24 kDa

SPECIFICATIONS

SDS-PAGE 24-27 kDa, reducing conditions

Activity Measured by its ability to cleave the fluorogenic peptide substrate, Mca-RPKPVE-Nval-WRK(Dnp)-NH₂ (Catalog # ES002).
The specific activity is >30,000 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 >85%, by SDS-PAGE under reducing conditions and visualized by Colloidal Coomassie® Blue stain at 5 μg per lane.

Formulation Supplied as a 0.2 μm filtered solution in Citrate, NaCl, CaCl₂, Benz, DTT, and Glycerol. See Certificate of Analysis for details.

Activity Assay Protocol

- Materials**
- Assay Buffer: 50 mM Tris, 10 mM CaCl₂, 150 mM NaCl, 0.05% (w/v) Brij35, pH 8.0
 - Recombinant Mouse Active Trypsin 3/PRSS3 (rmTrypsin 3) (Catalog # 7235-SE)
 - Substrate: MCA-Arg-Pro-Lys-Pro-Val-Glu-NVAL-Trp-Arg-Lys-(DNP)-NH₂ (Catalog # ES002), 2 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 rmTrypsin 3 to 0.007 ng/μL in Assay Buffer.
 2. Dilute Substrate to 20 μM in Assay Buffer.
 3. Load into a plate 50 μL of 0.007 ng/μL rmTrypsin 3, and start the reaction by adding 50 μL of 20 μM Substrate. For Substrate Blanks, load 50 μL of Assay Buffer and 50 μL of 20 μM Substrate.
 4. Read plate at excitation and emission wavelengths of 320 nm and 405 nm, respectively, in kinetic mode for 5 minutes.
 5. Calculate specific activity:

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

*Adjusted for Substrate Blank

**Derived using calibration standard MCA-Pro-Leu-OH (Bachem, Catalog # M-1975).

- Final Assay Conditions**
- Per Well:
- rmTrypsin 3: 0.35 ng (0.00035 μg)
 - Substrate: 10 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, -70 °C as supplied.
- 3 months, -70 °C under sterile conditions after opening.

BACKGROUND

Mouse Trypsin 3, encoded by the Prss3 gene, is also known as mesotrypsin and brain trypsin (1). It is one of three trypsin isoforms produced by the pancreas (2). It is secreted into the duodenum lumen, where it is activated by enterokinase. Trypsin 3 differs from Trypsin 1 and 2 because it is resistant to polypeptide trypsin inhibitors such as the Kunitz-type soybean trypsin inhibitor and the Kazal-type pancreatic secretory trypsin inhibitor (3). It has been proposed that a function of Trypsin 3 is the degradation of trypsin inhibitors to facilitate the digestion of foods rich in these proteins (4). Recombinant mouse Trypsin 3 is expressed and processed from *E. coli*, which yields the purified mature chain of mouse Trypsin 3.

References:

1. Nyarahucha, C.N.M. *et al.* (1997) J. Biol. Chem. **272**:10573.
2. Rinderknecht, H. *et al.* (1984) Gastroenterology **86**:681.
3. Katona, G. *et al.* (2002) J. Mol. Biol. **315**:1209.
4. Szmola, R. *et al.* (2003) J. Biol. Chem. **278**:48580.