Recombinant Human Trypsin 3/PRSS3

Catalog Number: 3710-SE

DESCRIPTION

Source	Mouse myeloma cell line, NS0-derived Val16-Ser247, with a C-terminal 10-His tag Accession # NP_002762
N-terminal Sequence Analysis	Val16
Structure / Form	Pro form
Predicted Molecular Mass	27 kDa

SPECIFICATIONS

SDS-PAGE	32 kDa, reducing conditions
Activity	Measured by its ability to cleave the fluorogenic peptide substrate, Mca-RPKPVE-Nval-WRK(Dnp)-NH_2 (Catalog # ES002). The specific activity is >4,000 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	>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 MES and NaCl. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Activation Buffer: 50 mM Tris, 10 mM $CaCl_2$, 150 mM NaCl, 0.05% (w/v) Brij-35, pH 7.5 (TCNB)
- Assay Buffer: 50 mM Tris, 10 mM $CaCl_2$, 150 mM NaCl, 0.05% (w/v) Brij-35, pH 8.0
- Recombinant Human Trypsin 3/PRSS3 (rhTrypsin 3) (Catalog # 3710-SE)
- Recombinant Human Enteropeptidase/Enterokinase (rhEnterokinase) (Catalog # 1585-SE)
- Bacterial Thermolysin (Thermolysin) (Catalog # 3097-ZN)
- Substrate: Mca-Arg-Pro-Lys-Pro-Val-Glu-NVAL-Trp-Arg-Lys(Dnp)-NH_2 (Catalog # ES002)
- 1,10 Phenanthroline (Sigma, Catalog # 320056), 0.6 M in DMSO
- F16 Black Maxisorp Plate (Nunc, Catalog # 475515)
- Fluorescent Plate Reader (Model: SpectraMax Gemini EM by Molecular Devices) or equivalent

Assay

1. Activate rhEnterokinase with Thermolysin.
   a. Dilute rhEnterokinase to 100 µg/mL with Activation Buffer.
   b. Dilute Thermolysin to 3.16 µg/mL with Activation Buffer.
   c. Mix equal volumes of diluted rhEnterokinase with Thermolysin
   d. Incubate at 37 °C for 30 minutes.
   e. Stop the reaction by adding an equal volume of 20 mM 1,10 Phenanthroline.
2. Activate rhTrypsin 3, with activated rhEnterokinase.
   a. Dilute the activated rhEnterokinase to 2 µg/mL in Assay Buffer.
   b. Dilute rhTrypsin 3 to 200 µg/mL in Assay Buffer.
   c. Mix equal volumes of the diluted rhEnterokinase and diluted rhTrypsin 3.
   d. Incubate at 37 °C for 90 minutes.
3. After incubation, dilute activated rhTrypsin 3 to 0.1 µg/mL in Assay Buffer.
4. Dilute Substrate to 20 µM in Assay Buffer.
5. Load in a black well plate 50 µL of rhTrypsin 3 at 0.1 µg/mL, and start the reaction by adding 50 µL of 20 µM Substrate. Include a Substrate Blank containing 50 µL of Assay Buffer and 50 µL of 20 µM Substrate.
6. Read at excitation and emission wavelengths of 320 nm and 405 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 Mca-Pro-Leu-OH (Bachem, Catalog # M-1975).

Final Assay Conditions

Per Well:

- rhTrypsin 3: 0.005 µg
- Substrate: 10 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.

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BACKGROUND

Human Trypsin 3, encoded by the PRSS3 gene, is also known as mesotrypsin (1). Constituting less than 10% of the total trypsin content in normal pancreatic juice, it is one of the three trypsin isoforms produced by the pancreas (2). Compared to Trypsin 1 and 2, one intriguing feature of Trypsin 3 is its resistance to polypeptide trypsin inhibitors, such as the Kunitz-type soybean trypsin inhibitor or the Kazal-type pancreatic secretory trypsin inhibitor. As revealed by the crystal structure, this resistance is likely due to the presence of an arginine residue in place of the highly conserved Gly198 (3). Trypsin 3 is synthesized in the pancreas and secreted into the duodenum lumen, where it is activated by enterokinase. One physiologic function of Trypsin 3 has been proposed to be degradation of trypsin inhibitors, which facilitates the digestion of those foods rich in these proteins (4).

References:

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3. Katona, G. *et al.* (2002) J. Mol. Biol. **315**:1209.
4. Szmola, R. *et al.* (2003) J. Biol. Chem. **278**:48580.

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