**DESCRIPTION**

**Source**
Mouse myeloma cell line, NS0-derived
Ile24-Ser247, with a C-terminal 10-His tag
Accession # NP_002762

**N-terminal Sequence Analysis**
Ile24

**Structure / Form**
Active form

**Predicted Molecular Mass**
26 kDa

**SPECIFICATIONS**

**SDS-PAGE**
20-24 kDa, non-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 >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**
>80%, by SDS-PAGE visualized with Silver Staining and quantitative densitometry by Coomassie® Blue Staining.

**Formulation**
Supplied as a 0.2 µm filtered solution in HCl, NaCl, CaCl₂ and Brij-35. See Certificate of Analysis for details.

**Activity Assay Protocol**

**Materials**
- Assay Buffer: 50 mM Tris, 0.15 M NaCl, 10 mM CaCl₂, 0.05% Brij-35 (w/v), pH 7.5 (TCNB)
- Recombinant Human Active Trypsin 3/PRSS3 (rhTrypsin 3) (Catalog # 3714-SE)
- Substrate MCA-Arg-Pro-Leu-0H (Bachem, Catalog # M-1975)
- F16 Black Maxisorp Plate (Nunc, Catalog # 475515)
- Fluorescent Plate Reader (Model: SpectraMax Gemini EM by Molecular Devices) or equivalent

**Assay**
1. Dilute rhTrypsin 3 to 0.06 µg/mL in Assay Buffer.
2. Dilute Substrate to 80 µM in Assay Buffer.
3. Load 50 µL of 0.06 µg/mL of rhTrypsin 3 into a plate, and start the reaction by adding 50 µL of 80 µM Substrate. Include a Substrate Blank containing 50 µL of Assay Buffer and 50 µL of 80 µM Substrate.
4. Read at excitation and emission wavelengths of 320 nm and 405 nm (top read), respectively in kinetic mode for 5 minutes.
5. Calculate specific activity:

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

   *Adjusted for Substrate Blank
   **Derived using calibration standard MCA-Pro-Leu-0H (Bachem, Catalog # M-1975).

**Final Assay Conditions**
Per Well:
- rhTrypsin 3 (Active form): 0.003 µg
- Substrate: 40 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.

**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 proposed physiological function of Trypsin 3 is degradation of trypsin inhibitors, which facilitates the digestion of those foods rich in these proteins (4).

**References:**