**DESCRIPTION**

**Source**
Mouse myeloma cell line, NS0-derived Ala16-Ser247, with a C-terminal 10-His tag
Accession # NP_002760

**N-terminal Sequence Analysis**
Ala16

**Structure / Form**
Pro form

**Predicted Molecular Mass**
26 kDa

**SPECIFICATIONS**

**SDS-PAGE**
32 kDa and 36 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 >3,000 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 under reducing conditions and visualized by silver stain.

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

**Activity Assay Protocol**

**Materials**
- Activation Buffer: 50 mM Tris, 0.15 M NaCl, 10 mM CaCl₂, 0.05% (w/v) Brij-35, pH 7.5 (TCNB)
- Assay Buffer: 100 mM Tris, 150 mM NaCl, 10 mM CaCl₂, 0.05% (w/v) Brij-35, pH 8.0
- Recombinant Human Trypsin 1/PRSS1 (rhTrypsin 1) (Catalog # 3848-SE)
- Recombinant Human Enteropontidase/Enterokinase (rhEnterokinase) (Catalog # 1585-SE)
- Bacterial Thermolysin (Thermolysin) (Catalog # 3097-ZN)
- Substrate: MCA-Arg-Pro-Lys-Pro-Lys-Pro-Val-Glu-Nval-Trp-Arg-Lys(DNP)-NH₂ (Catalog # ES002) 2 mM stock in DMSO
- 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 (see also R&D Systems, Catalog # 1585-SE).
   a. Activate rhEnterokinase at 50 μg/mL with 1.58 μg/mL Thermolysin in Activation Buffer.
   b. Incubate at 37 °C for 30 minutes.
   c. Stop the reaction with an equal volume of 20 mM of 1,10 Phenanthroline for a final concentration of 10 mM.

2. Activate rhTrypsin 1 at 100 μg/mL with activated rhEnterokinase at 0.4 μg/mL.
   a. Dilute the activated rhEnterokinase to 0.8 μg/mL in Assay Buffer.
   b. Dilute rhTrypsin 1 to 200 μg/mL in Assay Buffer.
   c. Mix equal volumes of 0.8 μg/mL rhEnterokinase with 200 μg/mL rhTrypsin 1.
   d. Incubate reaction at room temperature for 15 minutes.

3. Dilute activated rhTrypsin 1 to 0.1 μg/mL in Assay Buffer.
4. Dilute Substrate to 20 μM in Assay Buffer.
5. In a plate load 50 μL of 0.1 μM rhTrypsin 1 to wells, and start the reaction by adding 50 μL of 20 μM Substrate. Include a Substrate Blank containing 50 μL 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:

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

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

**Final Assay Conditions**

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<th>Per Well:</th>
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<td>rhTrypsin 1: 0.005 μg</td>
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<td>Substrate: 10 μM</td>
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**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.
Human Trypsin 1, encoded by the PRSS1 gene, is also known as cationic trypsinogen (1). Constituting approximately two-thirds of the total trypsin content in normal pancreatic juice, it is the most abundant trypsin isoform produced by the pancreas. It contains a signal peptide (residues 1-15), a pro region (residues 16-23), and a mature chain (residues 24-247). Trypsin 1 is synthesized in the pancreas and secreted into the duodenum lumen, where it is activated by enterokinase. Its major physiologic function is to digest food and to activate other pro-enzymes (2). Mutations in the PRSS1 gene can cause hereditary pancreatitis (3).

References: