

DESCRIPTION

Source *E. coli*-derived mouse Matriptase/ST14 protein
Gly596-Val855, with an N-terminal Met and 6-His tag
Accession # NP_035306
The protein was auto-activated and further purified.

N-terminal Sequence Analysis Met & Val615

Predicted Molecular Mass 3 kDa & 26 kDa

SPECIFICATIONS

SDS-PAGE 27 kDa (major) and minor auto-activation fragments, reducing conditions

Activity Measured by its ability to cleave the fluorogenic peptide substrate Boc-QAR-AMC (Catalog # ES014).
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 Tris and Glycerol. See Certificate of Analysis for details.

Activity Assay Protocol

- Materials**
- Assay Buffer: 50 mM Tris, 50 mM NaCl, 0.01% (v/v) Tween® 20, pH 9.0
 - Recombinant Mouse Matriptase/ST14 Catalytic Domain (rmMatriptase) (Catalog # 4735-SE)
 - Substrate: BOC-Gln-Ala-Arg-AMC (Catalog # ES014)
 - F16 Black Maxisorp Plate (Nunc, Catalog # 475515)
 - Fluorescent Plate Reader (Model: SpectraMax Gemini EM by Molecular Devices) or equivalent

- Assay**
1. Dilute rmMatriptase to 0.2 ng/μL in Assay Buffer.
 2. Dilute Substrate to 50 μM in Assay Buffer.
 3. Load into a plate 50 μL of 0.2 ng/μL rmMatriptase, and start the reaction by adding 50 μL of 50 μM Substrate. Include a Substrate Blank containing 50 μL of Assay Buffer and 50 μL of 50 μM Substrate.
 4. Read at excitation and emission wavelengths of 380 nm and 460 nm, respectively, in kinetic mode for 5 minutes.
 5. Calculate specific activity:

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

*Adjusted for Substrate Blank

**Derived using calibration standard 7-Amino, 4-Methyl Coumarin (Sigma, Catalog # A9891).

Final Assay Conditions Per Well:

- rmMatriptase: 0.010 μg
- Substrate: 25 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.

BACKGROUND

Matriptase, a mouse type II membrane serine protease encoded by the ST14 (suppression of tumorigenicity 14) gene, is also known as epithin, and membrane-type serine protease 1/MT-SP1 (1-2). Its human ortholog MT-SP1/Matriptase (Catalog # 3946-SE), which shares 81% amino acid identity with epithin, has been thought to play an important role in tumor biology and is a potential target for anti-cancer therapy (3). Matriptase has a multidomain structure containing a putative N-terminal transmembrane region, two CUB domains, four LDLRA repeats, and a C-terminal serine protease domain (1). The protease domain of epithin starts with Val615. R&D Systems recombinant mouse Matriptase is an active protease and consists of the catalytic domain (Val615 to Val855) and a short peptide (Gly596 to Arg614).

References:

1. Cho, E. *et al.* (2001) J. Biol. Chem. **276**:44581.
2. List, K. *et al.* (2006) Mol. Med. **12**:1.
3. Uhland, K. (2006) Cell Mol. Life Sci. **63**:2968.