Recombinant Human Matriptase/ST14 Catalytic Domain

Catalog Number: 3946-SE

DESCRIPTION

Source	*E. coli*-derived Gly596-Val855, with an N-terminal Met and 6-His tag Accession # NP_068813 The protein was purified, auto-activated and further purified.
N-terminal Sequence Analysis	Val615
Predicted Molecular Mass	26 kDa; Other fragments of 19 kDa, 17 kDa, 9 kDa and 6 kDa are also observed in the recombinant protein preparation.

SPECIFICATIONS

SDS-PAGE	27 kDa (major), 20 kDa, 17 kDa, 10 kDa, 6.5 kDa, reducing conditions
Activity	Measured by its ability to cleave the fluorogenic peptide substrate Boc-QAR-AMC (Catalog # ES014). The specific activity is >10,000 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	>90%, 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-HCl 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 Human Matriptase/ST14 Catalytic Domain (rhMatriptase) (Catalog # 3946-SE)
- Substrate: BOC-Gln-Ala-Arg-AMC (Catalog # ES014), 10 mM stock in DMSO
- F16 Black Maxisorp Plate (Nunc Cat. # 475515)
- Fluorescent Plate Reader (Model: SpectraMax Gemini EM by Molecular Devices) or equivalent

Assay

1. Dilute rhMatriptase to 0.1 µg/mL in Assay Buffer.
2. Dilute Substrate to 50 µM in Assay Buffer.
3. Load 50 µL of 0.1 µg/mL rhMatriptase into a plate, 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 Substrate.
4. Read at excitation and emission wavelengths of 380 nm and 460 nm (top read), respectively, in kinetic mode for 5 minutes.
5. Calculate specific activity:

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

*Adjusted for Substrate Blank

**Derived using calibration standard 7-Amino, 4-Methyl Coumarin (AMC) (Sigma, Cat. # A-9891).

Final Assay Conditions

Per Well:

- rhMatriptase: 0.005 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

Human matriptase, encoded by the ST14 (suppression of tumorogenicity 14) gene, is also known as tumor associated differentially expressed gene 15 protein/TADG-15), epithin, and membrane-type serine protease 1/MT-SP1 (1). Predicted to have a significant role in tumor biology, matriptase may be a novel target for anti-cancer therapy (2). However, expressed in most human epithelia, matriptase is also important in several physiological processes (1). For example, it activates prostasin to initiate a protease cascade that is essential for epidermal differentiation (3), and it converts a single-chain IGFBP-rp1 into the two-chain form (4).

Matriptase is a type II transmembrane serine protease with a complex modular structure (1). The 855 amino acid (aa) sequence of human matriptase consists of a cytoplasmic tail (aa 1-55), a transmembrane domain (aa 56-76), and an extracellular portion (aa 77-855). The latter contains the following domains: SEA (aa 86-201), two CUBs (aa 214-334 and 340-447), four LDLRAs (aa 452-486, 487-523, 524-560, and 566-603), and a serine protease (aa 615-855). The physiological activation of the single-chain zymogen requires the cleavage at the SEA domain within the ER or Golgi, association with HAI-1, which facilitates the transport of the protease to the cell surface, and auto-cleavage at QAR-V(615)VGG (1). The activated matriptase is inhibited by HAI-1, and the resulting HAI-1 complex can be shed from the cell surface (1). R&D Systems recombinant human (rh) ST14 corresponds to the catalytic domain, and is inhibited effectively by rhHAI-1 and rhHAI-2A (Catalog # 1048-PI and 1106-PI).

References:

1. List, K. *et al.* (2006) Mol. Med. **12**:1.
2. Uhland, K. (2006) Cell. Mol. Life Sci. **63**:2968.
3. Netzel-Arnett, S. *et al.* (2006) J. Biol. Chem. **281**:32941.
4. Ahmed, S. *et al.* (2006) FEBS J. **273**:615.

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