

DESCRIPTION

Source Mouse myeloma cell line, NS0-derived human Furin protein
Asp108-Glu715, with a C-terminal 10-His tag
Accession # P09958

N-terminal Sequence Analysis Asp108 & Asp131

Structure / Form Mature

Predicted Molecular Mass 67 kDa

SPECIFICATIONS

SDS-PAGE 58-85 kDa, reducing conditions

Activity Measured by its ability to cleave the fluorogenic peptide substrate pERTKR-AMC (Catalog # ES013).
The specific activity is >125 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, CaCl₂, NaCl, Brij-35 and Glycerol. See Certificate of Analysis for details.

Activity Assay Protocol

- Materials**
- Assay Buffer: 25 mM Tris, 1 mM CaCl₂, 0.5% (w/v) Brij-35, pH 9.0
 - Recombinant Human Furin (rhFurin) (Catalog # 1503-SE)
 - Substrate: p-Glu-Arg-Thr-Lys-Arg-AMC (Catalog # ES013)
 - F16 Black Maxisorp Plate (Nunc, Catalog # 475515)
 - Fluorescent Plate Reader (Model: SpectraMax Gemini EM by Molecular Devices) or equivalent

- Assay**
1. Dilute rhFurin to 4 μg/mL in Assay Buffer.
 2. Dilute Substrate to 100 μM in Assay Buffer.
 3. Load into a black well plate 50 μL of 4 μg/mL of rhFurin, and start the reaction by adding 50 μL of 100 μM Substrate. Include a Substrate Blank containing 50 μL of Assay Buffer and 50 μL of 100 μM 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:

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

*Adjusted for Substrate Blank

**Derived using calibration standard 7-amino, 4-Methyl Coumarin (Sigma, Catalog # A-9891).

- Final Assay Conditions**
- Per Well:
- rhFurin: 0.2 μg
 - Substrate: 50 μM

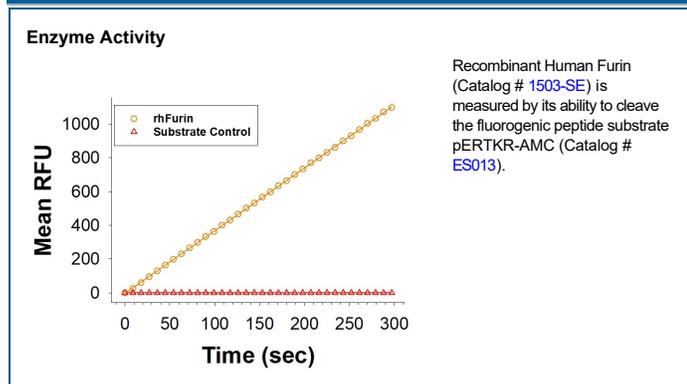
PREPARATION AND STORAGE

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.

DATA



BACKGROUND

Furin is a member of the proprotein convertase (PC) family, which belongs to the subtilisin superfamily of serine protease (1-3). As a cellular protease, Furin processes a variety of proproteins in secretory pathway compartments by cleaving after Arg-Xaa-Lys/Arg-Arg-like motifs, which usually reside at the end of the pro regions of these proproteins. Examples of the proprotein substrates are growth factors and receptors, extracellular matrix proteins, and other proteases. Furin has an essential role in embryogenesis and homeostasis and is implicated in various pathologies such as cancer, neurodegenerative diseases and anthrax. It is synthesized as a 794 amino acid type I transmembrane protein precursor with a signal peptide (residues 1-24), a pro region (residues 25-107), which play a crucial role in the folding, activation and transport of Furin, and a mature chain (residues 108-794) (1-3). The mature chain consists of the subtilisin-like catalytic domain, a P domain, which is essential for enzyme activity and the modulation of pH and calcium requirements, and a cytoplasmic domain, which controls the localization and sorting of Furin in the *trans*-Golgi network/endosomal system. The purified recombinant human Furin (residues 108-715) corresponds to the mature enzyme terminated before the transmembrane domain.

References:

1. Van den Ouweland, A.M. *et al.* (1990) *Nucleic Acids Res.* **18**:664.
2. Barr, P.J. *et al.* (1991) *DNA Cell Biol.* **10**:319.
3. Thomas, G. (2002) *Nature Rev. Mol. Cell Biol.* **3**:753.