Recombinant Human Furin

Catalog Number: 1503-SE

DESCRIPTION

Source	Mouse myeloma cell line, NS0-derived 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	65-85 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. See Activity Assay Protocol on www.RnDSystems.com.
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 Tris, $CaCl_2$, NaCl, Brij-35 and Glycerol. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 25 mM Tris, 1 mM $CaCl_2$, 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:

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

*Adjusted for Substrate Blank

**Derived using calibration standard 7-amino, 4-Methyl Coumarin (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.

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.

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