Recombinant Mouse Furin

Catalog Number: 6450-SE

DESCRIPTION

Source	Chinese Hamster Ovary cell line, CHO-derived mouse Furin protein Gln25-Glu714, with a C-terminal 10-His tag Accession # P23188
N-terminal Sequence Analysis	Asp108, Cys198, Ser239, Glu684
Predicted Molecular Mass	77 kDa

SPECIFICATIONS

SDS-PAGE	60-80 kDa, reducing conditions
Activity	Measured by its ability to cleave the fluorogenic peptide substrate pERTKR-AMC (Catalog # ES013). The specific activity is >150 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, $CaCl_2$, 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 Mouse Furin (rmFurin) (Catalog # 6450-SE)
- Substrate: p-Glu-Arg-Thr-Lys-Arg-AMC (Catalog # ES013), 8 mM in deionized water
- F16 Black Maxisorp Plate (Nunc, Catalog # 475515)
- Fluorescent Plate Reader (Model: Gemini EM by Molecular Devices) or equivalent

Assay

1. Dilute rmFurin to 2 µg/mL in Assay Buffer.
2. Dilute Substrate to 100 µM in Assay Buffer.
3. Load into a black well plate 50 µL of the 2 µg/mL rmFurin and start the reaction by adding 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:

- rmFurin: 0.1 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 proteases. 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 (1, 2). Mouse Furin is a 793 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 108-793) (2, 3). Mouse Furin displays ~94% homology to the human Furin sequence and >99% homology to the subtilisin-like catalytic domain (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 (1). The purified recombinant mouse Furin (residues 108-714) corresponds to the mature enzyme terminated before the transmembrane domain.

References:

1. Thomas, G. (2002) Nature Rev. Mol. Cell Biol. **3**:753.
2. Creemers, J.W. and W.J. Van de Ven. (2004) in Handbook of Proteolytic Enzymes (ed. Barrett, *et al.*), pp. 1531, Academic Press, San Diego.
3. Hatsuzawa, K. *et al.* (1990) J. Biol. Chem. **265**:22075.

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