Furin

50 units Lot: 0561405 Exp: 5/15
2,000 U/ml Store at –20°C

Recognition Site:
Arg-X-X-Arg

Description: Furin is a ubiquitous subtilisin-like proprotein convertase. It is the major processing enzyme of the secretory pathway and is localized in the trans-golgi network (1,2). Substrates of Furin include blood clotting factors, serum proteins and growth factor receptors such as the insulin-like growth factor receptor (3). The minimal cleavage site is Arg-X-(Lys/Arg)-Arg*. However, the enzyme prefers the site Arg-X-(Lys/Arg)-Arg*. An additional arginine at the P6 position appears to enhance cleavage (4). Furin is inhibited by EGTA, α1-Antitrypsin Portland (5) and polyarginine compounds (6).

Note: Both Furin and Onchocerca volvulus blisterase (NEB #P5204) will cleave peptide substrates with the sequence, Arg-X-(Lys/Arg)-Arg. However, the ability of either enzyme to cleave a particular protein substrate appears to depend on its tertiary structure as well as on the amino acids immediately surrounding the cleavage site (7).

Source: Isolated from Spodoptera frugiperda (Sf9) cells infected with recombinant baculovirus carrying truncated human furin (kindly provided by R. Fuller) (3).

Supplied in: 10 mM MES (pH 7.0 @ 25°C), 1 mM CaCl₂ and 50% glycerol. Store at –20°C.

Molecular Weight: The calculated molecular weight of truncated human furin is 52.7 kDa. Its apparent molecular weight in SDS-PAGE gels is 57 kDa (4).

Unit Definition: One unit is defined as the amount of Furin that will release 1 pmol of AMC from the fluorogenic peptide BOC-RVRR-AMC (Bachem #I-1645) in one minute (1 pmol of AMC/min) at 30°C.

Unit Assay Conditions: 100 mM HEPES (pH 7.5 @ 25°C), 0.5% Triton X-100, 1 mM CaCl₂, 1 mM 2-mercaptoethanol, 100 µM BOC-RVRR-AMC and enzyme in a 100 µl volume at 30°C.

Fusion Protein Digestion: One unit will cut 25 µg test substrate to 95% completion in 6 hours or less, while 0.5 units will cut 25 µg of test substrate to 95% completion in 16 hours or less.

Fusion Protein Digestion Conditions: Furin is added to 25 µg of an MBP fusion protein test substrate, MBP-αAsAl. The reaction is carried out in 25 µl, 100 mM Heps (pH 7.5 @25°C), 0.5% Triton X-100, 1 mM CaCl₂, 1 mM 2-mercaptoethanol at 25°C.

References:
1. van den Ouweland, A. M. W. et al. (1990) Nucleic Acid Res. 18, 664.

Note: Both Furin and blisterase (NEB #P5204) will cleave peptide substrates with the sequence, Arg-X-(Lys/Arg)-Arg. However, the ability of either enzyme to cleave a particular protein substrate appears to depend on its tertiary structure as well as on the amino acids immediately surrounding the cleavage site (7).

Source: Isolated from Spodoptera frugiperda (Sf9) cells infected with recombinant baculovirus carrying truncated human furin (kindly provided by R. Fuller) (3).

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Fusion Protein Digestion: One unit will cut 25 µg test substrate to 95% completion in 6 hours or less, while 0.5 units will cut 25 µg of test substrate to 95% completion in 16 hours or less.

Fusion Protein Digestion Conditions: Furin is added to 25 µg of an MBP fusion protein test substrate, MBP-αAsAl. The reaction is carried out in 25 µl, 100 mM Heps (pH 7.5 @25°C), 0.5% Triton X-100, 1 mM CaCl₂, 1 mM 2-mercaptoethanol at 25°C.

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