

DESCRIPTION

Source *E. coli*-derived
Ser38-Pro704, with an N-terminal Met and 6-His tag
Accession # Q9BYT8

N-terminal Sequence Analysis Met

Predicted Molecular Mass 77 kDa

SPECIFICATIONS

SDS-PAGE 73 kDa, reducing conditions

Activity Measured by its ability to cleave a fluorogenic peptide substrate, (7-methoxycoumarin-4-yl)acetyl-Pro-Leu-Gly-Pro-D-Lys(2,4-dinitrophenyl)-OH or Mca-PLGPK(Dnp)-OH.
The specific activity is >70 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 under reducing conditions and visualized by silver stain.

Formulation Supplied as a 0.2 μm filtered solution in Tris, NaCl and Glycerol. See Certificate of Analysis for details.

Activity Assay Protocol

- Materials**
- Assay Buffer: 25 mM Tris, 150 mM NaCl, pH 7.5
 - Recombinant Human Neurolysin (rhNeurolysin) (Catalog # 3814-ZN)
 - Substrate: MCA-Pro-Leu-Gly-Pro-D-Lys(DNP)-OH (Bachem, Catalog # M-2270), 2 mM stock in DMSO
 - F16 Black Maxisorp Plate (Nunc, Catalog # 475515)
 - Fluorescent Plate Reader (Model: SpectraMax Gemini EM by Molecular Devices) or equivalent

- Assay**
1. Dilute rhNeurolysin to 5 μg/mL in Assay Buffer.
 2. Dilute Substrate to 50 μM in Assay Buffer.
 3. Load into a black well plate 50 μL of 5 μg/mL rhNeurolysin, and start the reaction by adding 50 μL of 50 μM Substrate. Include a Substrate Blank containing 50 μL Assay Buffer and 50 μL of 50 μM Substrate.
 4. Read at excitation and emission wavelengths of 320 nm and 405 nm (top read), respectively, in kinetic mode for 5 minutes.
 5. Calculate specific activity:

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

*Adjusted for Substrate Blank

**Derived using calibration standard MCA-Pro-Leu-OH (Bachem, Catalog # M-1975).

- Final Assay Conditions** Per Well:
- rhNeurolysin: 0.25 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

Neurolysin, also known as Oligopeptidase M (mitochondrial peptidase), soluble angiotensin II-binding protein and endopeptidase EC 3.4.24.16 (1, 2), is a homologue of Thimet Oligopeptidase (THOP1), a zinc peptidase of the M3 family that also includes mitochondrial intermediate peptidase. Neurolysin expresses in two forms with the difference of an N-terminal transit peptide that targets the localization to mitochondria (1). The shorter form exists in cytosol. The recombinant human Neurolysin is expressed without the transit peptide and is isolated from the cytosol. Like THOP1, Neurolysin is capable of cleaving a number of vasoactive peptides such as bradykinin and neurotensin (3). All known substrates of THOP1 and Neurolysin contain 17 or fewer amino acids.

References:

1. Serizawa, A. *et al.* (1995) *J. Biol. Chem.* **270**:2092.
2. Barrett, A.J. and J.M. Chen (2004) in *Handbook of Proteolytic Enzymes* (ed. Barrett, A.J. *et al.*) pp. 356, Elsevier Academic Press, San Diego.
3. Norman, M.U. *et al.* (2003) *Am. J. Physiol. Heart Circ. Physiol.* **284**:H1978.