Recombinant Human Neprilysin/CD10

Catalog Number: 1182-ZN

Background

Neprilysin (NEP, neutral endopeptidase 24.11, EC 3.4.24.11) is a zinc metallopeptidase expressed at the cell surface of a variety of cells. The enzyme functions both as an endopeptidase with a thermolysin-like specificity and as a dipeptidylcarboxypeptidase. NEP has been shown to be involved in the degradation of enkephalins in the mammalian brain and the inactivation of circulating atrial natriuretic peptide (4, 5). NEP has also been identified as the common acute lymphoblastic leukemia antigen (CALLA), and to be expressed on the surface of lymphocytes in some disease states (2, 3). These and other observations have resulted in considerable clinical interest in NEP as a potential target for analgesics and antihypertensive drugs. NEP is also a major degrading enzyme of amyloid β peptide (Aβ) in the brain, indicating that down-regulation of NEP activity, which could be caused by aging, can contribute to the development of Alzheimer's disease by promoting Aβ accumulation (6).

References:

1. Malfroy, B. *et al.* FEBS Lett. **229:**206.
2. LeTarte, M. *et al.* (1988) J. Exp. Med. **168:**1247.
3. Shipp, M.A. *et al.* (1988) Proc. Natl. Acad. Sci. USA **85:**4819.
4. Malfroy, B. *et al.* (1978) Nature **276:**523.
5. Kenny, A.J. and S.L. Stephenson (1988) FEBS Lett. **232:**1.
6. Itwata, N. *et al.* (2001) Science **292:**1550.

Description

Source	*Spodoptera frugiperda*, *Sf* 21 (stably transfected)-derived Tyr45 - Trp743 Accession # CAA30157.1
N-terminal Sequence Analysis	Tyr45
Predicted Molecular Mass	80 kDa

Specifications

SDS-PAGE	82 kDa, reducing conditions
Activity	Measured by its ability to cleave the fluorogenic peptide substrate, Mca-RPPGFSAFK(Dnp)-OH, R&D Systems, Catalog # ES005. The specific activity is > 1200 pmoles/min/µg, as measured under the described conditions. See Activity Assay Protocol.
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 and NaCl. See Certificate of Analysis for details.

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.

Activity Assay Protocol

Materials

• Assay Buffer: 50 mM Tris, 0.05% Brij-35 (v/v), pH 9.0

• Recombinant human Neprilysin (R&D Systems, Catalog # 1182-ZN)

• Substrate: MCA-Arg-Pro-Pro-Gly-Phe-Ser-Ala-Phe-Lys(DNP)-OH (R&D Systems, Catalog # ES005) Prepare 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 rhNeprilysin to 0.1 µg/mL in Assay Buffer.
2. Dilute Substrate to 20 µM in Assay Buffer.
3. Load in plate 50 µL of 0.1 µg/mL of rhNeprilysin and start the reaction by adding 50 µL of 20 µM Substrate. As a Substrate Blank combine 50 µL of Substrate and 50 µL of Assay Buffer.
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:

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

*Adjusted for Substrate Blank

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

Final Assay Conditions Per Well

• rhNeprilysin: 0.005 µg

• Substrate: 10 µM

7/10/2009

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NOT FOR USE IN HUMANS.