

DESCRIPTION

Source Human embryonic kidney cell, HEK293-derived Neprilysin/CD10 protein
Tyr52-Trp750
Accession # XP_005546216.1
with an N-terminal 6-His tag

N-terminal Sequence Analysis His

Predicted Molecular Mass 81 kDa

SPECIFICATIONS

SDS-PAGE 86-95 kDa, under reducing conditions

Activity Measured by its ability to cleave the fluorogenic peptide substrate, Mca-RPPGFSAFK(Dnp)-OH (Catalog # ES005).
The specific activity is >1200 pmol/min/μg, as measured under the described conditions.

Endotoxin Level <0.10 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, NaCl and ZnCl₂. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 50 mM Tris, 0.5 M NaCl, pH 9.0
- Recombinant Cynomolgus/Rhesus Neprilysin (rcyno/rhesusNeprilysin) (Catalog # 10834-ZN)
- Substrate: Mca-Arg-Pro-Pro-Gly-Phe-Ser-Ala-Phe-Lys(DNP)-OH (Catalog # ES005), 2 mM stock in DMSO
- F16 Black Maxisorp Plate (Nunc, Catalog # 475515)
- Plate Reader (Model: SpectraMax Gemini EM by Molecular Devices) or equivalent

Assay

1. Dilute rcyno/rhesusNeprilysin to 0.2 μg/mL in Assay Buffer.
2. Dilute Substrate to 60 μM in Assay Buffer.
3. Load into a plate 50 μL of 0.2 μg/mL rcyno/rhesusNeprilysin and start the reaction by adding 50 μL of 60 μM Substrate. Include a Substrate Blank containing 50 μL of Assay Buffer and 50 μL of 60 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:

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

*Adjusted for Substrate Blank

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

Final Assay Conditions

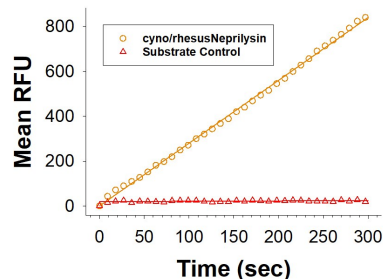
- Per Well:
- rcyno/rhesusNeprilysin: 0.01 μg
 - Substrate: 30 μM

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.

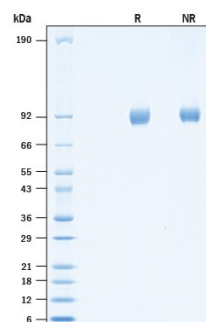
DATA

Enzyme Activity



Recombinant Cynomolgus Monkey/Rhesus Macaque Neprilysin/CD10 His-tag Protein Enzyme Activity
Recombinant Cynomolgus Monkey/Rhesus Macaque Neprilysin His-tag Protein (Catalog # 10834-ZN) is measured by its ability to cleave the fluorogenic peptide substrate, Mca-RPPGFSAFK(Dnp)-OH (Catalog # ES005).

SDS-PAGE



Recombinant Cynomolgus Monkey/Rhesus Macaque Neprilysin/CD10 His-tag Protein SDS-PAGE. 2 µg/lane of Recombinant Cynomolgus Monkey/Rhesus Macaque Neprilysin/CD10 His-tag Protein (Catalog # 10834-ZN) was resolved with SDS-PAGE under reducing (R) and non-reducing (NR) conditions and visualized by Coomassie® Blue staining, showing bands at 86-95 kDa.

BACKGROUND

Neprilysin/CD10, also known as NEP, neutral endopeptidase, enkephalinase, and common acute lymphocytic leukemia antigen (CALLA), is a zinc-dependent metallopeptidase type II integral membrane protein of the M13 subfamily of neutral endopeptidases. The enzyme functions both as an endopeptidase with a thermolysin-like specificity and as a dipeptidylcarboxypeptidase. It cleaves and degrades a variety of bioactive peptides including natriuretic peptides, enkephalins, and tachykinins at the amino side of hydrophobic residues. Neprilysin is widely distributed in tissues and highly conserved among mammals (1). Neprilysin is a noncovalently associated homodimer where the monomer is composed of a short intracellular domain, transmembrane helix, and large C-terminal extracellular domain including two domains that form a central cavity containing the active site (2). Although a membrane protein, an alternatively processed soluble form has been documented in circulation and retains similar enzymatic activity to the membrane bound Neprilysin (3). The soluble form is of interest as a prognostic biomarker for heart failure (4,5) and inhibition of Neprilysin is a therapeutic strategy for heart failure (6). Neprilysin was found to be a major degrading enzyme of amyloid beta peptide (A beta) in the brain, making it an important target in Alzheimer's disease studies (7). It metabolizes sensory and inflammatory neuropeptides making it a target for pain perception (8) and is an important marker for some types of lymphomas (9,10). There is also interest in Neprilysin as a biomarker (11) and as a therapeutic inhibition target for treatment of type 2 diabetes (12).

References:

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