Recombinant Human Neprilysin/CD10  
Catalog Number: 1182-ZNC

DESCRIPTION

Source  
Chinese Hamster Ovary cell line, CHO-derived  
Tyr52-Trp750, with an N-terminal 6-His tag  
Accession # P08473

N-terminal Sequence Analysis  
His

Predicted Molecular Mass  
81 kDa

SPECIFICATIONS

SDS-PAGE  
102 kDa, reducing conditions

Activity  
Measured by its ability to cleave the fluorogenic peptide substrate, Mca-RPPGFSAFK(Dnp)-OH (Catalog # ES005).  
The specific activity is >1,500 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 ZnCl₂. See Certificate of Analysis for details.

Activity Assay Protocol

Materials  
- Assay Buffer: 50 mM Tris, 0.05% Brij-35, pH 9.0  
- Recombinant Human Neprilysin (rhNeprilysin) (Catalog # 1182-ZNC)  
- Substrate: MCA-Arg-Pro-Pro-Gly-Phe-Ser-Ala-Phe-Lys(DNP)-OH (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 fluorogenic peptide Substrate to 20 µM in Assay Buffer.  
3. Load in a black well plate 50 µL of 0.1 µg/mL of rhNeprilysin (5 ng/well), 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:  
   \[ \text{Specific Activity (pmol/min/µg)} = \ \frac{\text{Adjusted } V_{max}^* \times \text{(RFU/min) \times Conversion Factor**}}{\text{amount of enzyme (µg)}} \]

   *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

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

Neprilysin/CD10, also known as NEP and neutral endopeptidase 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 (1, 2). NEP has also been identified as the common acute lymphocytic leukemia antigen (CALLA), and is expressed on the surface of lymphocytes in some disease states (3, 4). These and other observations have resulted in considerable interest in NEP as a 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 (5).

References:

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