Recombinant Human ECE-1
Catalog Number: 1784-ZN

DESCRIPTION

Source: Mouse myeloma cell line, NS0-derived
Gln90-Trp770, with an N-terminal signal peptide, 10-His tag and Val
Accession # P42892

N-terminal Sequence Analysis: His

Predicted Molecular Mass: 79 kDa

SPECIFICATIONS

SDS-PAGE: 115 kDa, reducing conditions

Activity: Measured by its ability to cleave the fluorogenic peptide substrate, Mca-RPPGFSAFK(Dnp)-OH (Catalog # ES005).
The specific activity is >2,500 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: 0.1 M MES, 0.1 M NaCl, pH 6.0
- Recombinant Human ECE-1 (rECE-1) (Catalog # 1784-ZN)
- Fluorogenic Peptide Substrate V: MCA-Arg-Pro-Pro-Gly-Phe-Ser-Ala-Phe-Lys(DNP)-OH (Catalog # ES005)
- F16 Black Maxisorp Plate (Nunc, Catalog # 475515)
- Fluorescent Plate Reader (Model: SpectraMax Gemini EM by Molecular Devices) or equivalent

Assay:
1. Dilute rECE-1 to 0.1 µg/mL in Assay Buffer.
2. Dilute Substrate to 20 µM in Assay Buffer.
3. Load into a black well plate 50 µL of 0.1 µg/mL of rECE-1, and start the reaction by adding 50 µL of 20 µM Substrate. Include a Substrate Blank containing 50 µL Assay Buffer and 50 µL of 20 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:

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

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

Final Assay Conditions: Per Well:
- rECE-1: 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

Endothelin-converting Enzyme 1 (ECE-1) is a zinc protease of the neprilysin (NEP) family, which also includes ECE-2, PEX, XCE, DINE, Kell and several NEP-like proteins (1). ECE-1 is a type II transmembrane protein with a short cytoplasmic tail and a large ectodomain. Four alternatively spliced isoforms differ in their cytoplasmic tail (2, 3). In addition to big endothelin-1, ECE-1 cleaves a variety of bioactive peptides such as bradykinin, neurotensin, angiotensin I, and substance P (1). Together with ECE-2, it is also involved in degradation of β-amyloid peptide (4). The ectodomain of human ECE-1, which is common to all isoforms, was expressed with an N-terminal His tag and purified.

References: