Recombinant Human ACE/CD143 Somatic Form

Catalog Number: 929-ZN

DESCRIPTION

Source	Mouse myeloma cell line, NS0-derived Leu30-Leu1261, with a C-terminal 10-His tag Accession # P12821.1
N-terminal Sequence Analysis	Leu30
Structure / Form	Recombinant Human ACE/CD143 Somatic Form is prone to proteolytic cleavage at C-terminus. The predominant form of the purified protein lacks the His tag.
Predicted Molecular Mass	143 kDa

SPECIFICATIONS

SDS-PAGE	160-180 kDa, reducing conditions
Activity	Measured by its ability to cleave the fluorogenic peptide substrate, Mca-RPPGFSAFK(Dnp)-OH (Catalog # ES005). The specific activity is >1,000 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, $ZnCl_2$ and Glycerol. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 50 mM MES, pH 6.5
- Recombinant Human ACE/CD143 Somatic Form (rhACE) (Catalog # 929-ZN)
- Substrate: 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 rhACE to 0.4 ng/µL in Assay Buffer.
2. Dilute Substrate to 20 µM in Assay Buffer.
3. In a plate load 50 µL of 0.4 ng/µL rhACE and start the reaction by adding 50 µL of 20 µM Substrate to the wells. Include a Substrate Blank containing 50 µL Assay Buffer and 50 µL 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:

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

*Adjusted for Substrate Blank

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

Final Assay Conditions

Per Well:

- rhACE: 0.020 µg
- Substrate: 10 µM

PREPARATION AND STORAGE

Shipping	The product is shipped with dry ice or equivalent. 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.

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BACKGROUND

ACE (also known as peptidyl-dipetidase A) is a zinc metallopeptidase important for blood pressure control and water and salt metabolism (2). It cleaves the C-terminal dipeptide from angiotensin I to produce the potent vasopressor octapeptide angiotensin II and inactivates bradykinin by the sequential removal of two C-terminal dipeptides. In addition to the two physiological substrates, ACE cleaves C-terminal dipeptides from various oligopeptides with a free C-terminus. Because of its location and specificity, ACE plays additional roles in immunity, reproduction and neuropeptide regulation. For example, ACE degrades Alzheimer amyloid β-peptide (Aβ), retards Aβ aggregation, deposition, fibril formation, and inhibits cytotoxicity (3).

ACE is a type I membrane protein and exists in two isoforms (2). Somatic ACE, found in endothelial, epithelial and neuronal cells, comprises two highly similar domains called N- and C-domains, each of which contains the HExxH consensus sequence for zinc binding. Germinal ACE, found exclusively in the testes, comprises a single catalytically active domain identical to the C-domain of somatic ACE except for an N-terminal 67 residue germinal ACE-specific sequence. Physiological functions of the two tissue-specific isozymes are not interchangeable (4). For example, sperm-specific expression of the germinal ACE, not the somatic ACE, in ACE knockout male mice restored fertility.

Soluble ACE is present in many biological fluids, such as serum, seminal fluid, amniotic fluid and cerebrospinal fluid (2). The soluble ACE is derived from the membrane forms by actions of secretases or sheddases. The identities of the secretases have not been revealed, although they belong to the family of zinc metallopeptidases (5, 6).

References:

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