Recombinant Human
Carboxypeptidase E/CPE
Catalog Number: 3587-ZN

DESCRIPTION

Source: Mouse myeloma cell line, NS0-derived Arg42-Ser453, with a C-terminal 6-His tag
Accession # NP_001864

N-terminal Sequence Analysis: Arg42
Structure / Form: Mature form
Predicted Molecular Mass: 47 kDa

SPECIFICATIONS

SDS-PAGE: 57 kDa, reducing conditions
Activity: Measured by its ability to cleave a peptide substrate, benzoyl-AR-OH. The product, Arg, is reacted with ortho-phthalaldehyde (OPA) to form a fluorescent molecule. The specific activity is >12,000 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 MES and NaCl. See Certificate of Analysis for details.

Activity Assay Protocol

Materials
- Assay Buffer: 50 mM NaOAc, 5 μM ZnCl₂, pH 5.5
- Recombinant Human Carboxypeptidase E/CPE (rhCPE) (Catalog # 3587-ZN)
- Substrate: Benzoyl-Ala-Arg-OH (Bachem, Catalog # G4145), 50 mM stock in DMSO
- ortho-phthalaldehyde (OPA) (134.13 g/mol) (Sigma, Catalog # P6657), 50 mg/mL stock in DMSO
- OPA Buffer: 0.2 M NaOH containing 0.1% mercaptoethanol (v/v)
- F16 Black Maxisorp Plate (Nunc, Catalog # 475515)
- Fluorescent Plate Reader (Model: SpectraMax Gemini EM by Molecular Devices) or equivalent

Assay
1. Dilute rhCPE to 0.1 ng/µL in Assay Buffer.
2. Dilute Substrate to 1 mM in Assay Buffer.
3. Combine 75 µL of 0.1 ng/µL rhCPE and 75 µL of 1 mM Substrate. Include a Blank containing 75 µL of Substrate only.
4. Incubate at room temperature for 10 minutes.
5. Add 150 µL of 15 mM OPA in 0.2 M NaOH containing 0.1% mercaptoethanol (v/v) to reaction vials and the Blank (stops reaction).
6. After stopping the reaction, add 75 µL of 0.1 ng/µL rhCPE to the Blank.
7. Mix well and incubate at room temperature for 2-10 minutes.
8. Load 200 µL of sample(s) and blank into plate.
9. Read at excitation and emission wavelengths of 330 nm and 450 nm (top read), respectively in endpoint mode.
10. Calculate specific activity:

Specific Activity (pmol/min/µg) = \frac{Adjusted Fluorescence* (RFU) \times Conversion Factor** (pmol/RFU)}{Incubation time (min) \times amount of enzyme (µg)}

*Ajusted for Substrate Blank
**Derived using calibration standard L-Arginine (Sigma, Catalog # A-5006).

Final Assay Conditions
Per Well:
- rhCPE: 0.005 µg
- Substrate: 0.25 mM
- OPA: 7.5 mM

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.
Encoded by the CPE gene and also known as Carboxypeptidase H, CPE is a single chain peptidase with an optimal pH range between 5.0-6.0. It is a zinc metallocarboxypeptidase that removes basic amino acids from the C-terminus of peptides (1). Like other metallocarboxypeptidases, its activity is stimulated by millimolar concentrations of Co²⁺. Its activity is regulated by pH-induced aggregation above pH 6.0. Its major function seems to process numerous peptide hormones and neurotransmitters. In addition to its proteolytic function, it also plays a role as a sorting receptor (2), which may be attributed to the sorting of this protein into the secretory pathway. The C-terminal domain of CPE causes the peripheral association of CPE with membranes below neutral pH, resulting in the association of this protein into membranes (3). CPE knockout mice live but become obese due to impaired glucose clearance and insulin resistance (4).

References: