Recombinant Human Carboxypeptidase M
Catalog Number: 7457-ZN

DESCRIPTION

Source
Chinese Hamster Ovary cell line, CHO-derived
Leu18-Ser423, with an N-terminal human CD33 signal sequence and a C-terminal 6-His tag
Accession # P14384

N-terminal Sequence Analysis
Leu18

Predicted Molecular Mass
47 kDa

SPECIFICATIONS

SDS-PAGE
50-56 kDa, reducing conditions

Activity
Measured by its ability to release L-arginine from Benzoyl-Ala-Arg, with detection of the arginine amino group by o-phthaldialdehyde.
The specific activity is >40,000 pmol/min/μg, as measured under the described conditions. See Activity Assay Protocol on www.RnDSystems.com

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 Colloidal Coomassie® Blue stain at 5 μg per lane.

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 MES, 0.2% Triton® X-100, 5 mM CaCl₂, pH 6.0
- Recombinant Human Carboxypeptidase M (rhCPM) (Catalog # 7457-ZN)
- Substrate: Bz-Ala-Arg-OH (Bachem, Catalog # G-4145), 50 mM stock in DMSO
- 2-Mercaptoethanol (Sigma, Catalog # M7154)
- NaOH, 2 M stock in deionized water
- o-Phthaldialdehyde (OPA) (Sigma, Catalog # P0657), 0.373 M in DMSO
- F16 Black Maxisorp Plate (Nunc, Catalog # 475515)
- Fluorescent Plate Reader (Model: SpectraMax Gemini EM by Molecular Devices) or equivalent

Assay
1. Dilute rhCPM to 0.04 μg/mL in Assay Buffer.
2. Dilute Substrate to 1 mM in Assay Buffer.
3. Mix (in duplicate) 150 μL of 0.04 μg/mL rhCPM and 150 μL 1 mM Substrate for a final concentration of 0.02 μg/mL and 500 μM respectively. Include controls containing 150 μL of 1 mM Substrate only. Incubate for 10 minutes at room temperature.
4. Stop reaction by adding 300 μL of a solution containing 15 mM o-PA in 0.2 M NaOH containing 0.1% (v/v) 2-Mercaptoethanol and mix well.
5. Add 150 μL of 0.04 μg/mL rhCPM to controls after stopping the reaction.
6. Incubate all for 10 minutes at room temperature.
7. Load 200 μL of the incubated samples in triplicate into the plate.
8. Read at excitation and emission wavelengths of 330 nm and 450 nm (top read), respectively, in endpoint mode.
9. Calculate Specific Activity:

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

**Derived using calibration standard L-Arginine (Sigma, Catalog # A5006).

Final Assay Conditions
- rhCPM: 0.002 μg
- Substrate: 0.25 mM
- o-PA: 7.5 mM

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
Carboxypeptidase M (CPM) is a zinc metallopeptidase specific for the removal of arginine and lysine residues from peptides. CPM is bound to the plasma membrane through a glycosylphosphatidylinositol anchor (1). The enzyme is thought to regulate the actions of some peptide hormones at the cell surface through their degradation (2). CPM is a biomarker for well-differentiated liposarcomas (3) and is also a marker for the maturation of monocytes to macrophages (4). CPM binds to the kinin B1 receptor on the cell surface, forming a complex that potentiates the signaling ability of the kinin B1 receptor (5). Recombinant human CPM was expressed as a C-terminally truncated protein to prevent the formation of the glycosylphosphatidylinositol anchor, resulting in the secretion of the protein.

References:

PRODUCT SPECIFIC NOTICES
Coomassie is a registered trademark of Imperial Chemical Industries Ltd. Triton is a registered trademark of Union Carbide Corp.