

DESCRIPTION

Source Mouse myeloma cell line, NS0-derived human Cathepsin A/Lysosomal Carboxypeptidase A protein
Ala29-Tyr480, with a C-terminal 10-His tag
Accession # P10619

N-terminal Sequence Analysis Ala29

Structure / Form Pro form

Predicted Molecular Mass 53 kDa

SPECIFICATIONS

SDS-PAGE 51-62 kDa, reducing conditions

Activity Measured by its ability to cleave the fluorogenic peptide substrate, Mca-RPPGFSAFK(Dnp)-OH (Catalog # ES005).
The specific activity is >75 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 and NaCl. See Certificate of Analysis for details.

Activity Assay Protocol

- Materials**
- Activation Buffer: 25 mM MES, 5 mM DTT, pH 6.0
 - Assay Buffer: 25 mM MES, 5 mM DTT, pH 5.5
 - Recombinant Human Cathepsin A/Lysosomal Carboxypeptidase A (rhCathepsin A) (Catalog # 1049-SE)
 - Recombinant Human Cathepsin L (rhCathepsin L) (Catalog # 952-CY)
 - E 64 (Tocris Catalog # 5208) , 50 mM stock in DMSO
 - 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 rhCathepsin A to 100 μg/mL in Activation Buffer.
 2. Dilute rhCathepsin L to 10 μg/mL in Activation Buffer.
 3. Combine equal volumes of rhCathepsin A and rhCathepsin L for final concentrations of 50 μg/mL and 5 μg/mL, respectively.
 4. Incubate reaction at 37 °C for 30 minutes.
 5. Stop reaction by adding E-64 to a final concentration of 10 μM.
 6. Dilute activated rhCathepsin A to 2.0 ng/μL in Assay Buffer.
 7. Dilute Substrate to 20 μM in Assay Buffer.
 8. Load 50 μL of 2.0 ng/μL rhCathepsin A into a plate, 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.
 9. Read at excitation and emission wavelengths of 320 nm and 405 nm (top read), respectively, in kinetic mode for 5 minutes.
 10. Calculate specific activity:

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

*Adjusted for Substrate Blank

**Derived using calibration standard MCA-Pro-Leu-OH (Bachem, Catalog # M-1975)

Final Assay Conditions Per Well:

- rhCathepsin A: 0.1 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

Cathepsin A/lysosomal carboxypeptidase A is a member of the serine carboxypeptidase family (1). Cathepsin A is a multifunctional enzyme that expresses deaminidase and esterase activities at neutral pH and carboxypeptidase activity at acidic pH. Also known as protective protein, its association with β -galactosidase (β -gal) and neuraminidase is essential for β -gal stability and neuraminidase activation in the lysosomes. Inherited deficiency of Cathepsin A causes the lysosomal storage disorder galactosialidosis, characterized by a combined secondary deficiency of β -gal and neuraminidase. Cathepsin A is capable of hydrolyzing a variety of bioactive peptide hormones including tachykinins, indicating that extralysosomal Cathepsin A plays a role in regulation of functions of these molecules (2). Cathepsin A is synthesized as a single-chain precursor and processed into heavy (32 kDa) and light (20 kDa) chains, which are linked by disulfide bonds.

References:

1. Pshezhetsky, A.V. (2004) in *Handbook of Proteolytic Enzymes* (ed. Barrett, A.J. et al.) p. 1923, Academic Press, San Diego.
2. Hiraiwa, M. (1999) *Cell. Mol. Life. Sci.* **56**:894.