Genorise® Recombinant Mouse Cathepsin C

Catalog Number: GR124146

Background

Cathepsin C (CTSC) also known as dipeptidyl peptidase I (DPP-I) is a lysosomal exo-cysteine protease belonging to the peptidase C1 family. In humans, it is encoded by the CTSC gene.[1] Cathepsin C appears to be a central coordinator for activation of many serine proteases in immune/inflammatory cells. Cathepsin C catalyses excision of dipeptides from the N-terminus of protein and peptide substrates, except if (i) the amino group of the N-terminus is blocked, (ii) the site of cleavage is on either side of a proline residue, (iii) the N-terminal residue is lysine or arginine, or (iv) the structure of the peptide or protein prevents further digestion from the N-terminus. Unlike the other members of the papain family, mature cathepsin C consists of four subunits, each composed of the N-terminal proregion fragment, the heavy chain and the light chain. Both the pro-region fragment and the heavy chain are glycosylated. Defects in the encoded protein have been shown to be a cause of Papillon-Lefevre disease.[2][3] Cathepsin C functions as a key enzyme in the activation of granule serine peptidases in inflammatory cells, such as elastase and cathepsin G in neutrophils cells and chymase and tryptase in mast cells. In many inflammatory diseases, such as rheumatoid arthritis, COPD, inflammatory bowel disease, asthma, sepsis, and cystic fibrosis, a significant portion of the pathogenesis is caused by increased activity of some of these inflammatory proteases. Once activated by cathepsin C, the proteases are capable of degrading various extracellular matrix components, which can lead to tissue damage and chronic inflammation.

References

1. Paris A, et al. (1995). FEBS Letters. 369 (2-3): 326–30.
2. Wani AA, et al. (2006). Journal of Periodontology. 77 (2): 233–7.
3. Meade JL, et al. (2006). Blood. 107 (9): 3665–8.

Genorise® Recombinant Mouse Cathepsin C

Catalog Number: GR124146

Description
Source: *E. coli* derived
Components: Asp25-Leu462
Accession # P97821
Predicted Molecular Mass: 24 kDa (monomer)

Specifications
Activity Measured by its ability to cleave the fluorogenic peptide substrate, Gly-Arg-7-amido-4-methylcoumarin (GR-AMC). The specific activity is > 6,000 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 SDSPAGE under reducing conditions and visualized by silver stain.
Formulation: Lyophilized from a 0.2 µm filtered solution in MES, NaCl and Glycerol.

Preparation and Storage
Reconstitution: Purified recombinant Mouse CATHEPSIN Cβ1 is an extremely hydrophobic protein that adheres strongly to surfaces. To ensure recovery, reconstitute at 20 µg/mL in sterile 4 mM HCl containing 1 mg/ml of human or Mouse serum albumin.
Shipping The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below.
Stability & Storage: Use a manual defrost freezer and avoid repeated freeze thaw cycles.

- 3 months -20 to -70°C as provided.
- 1 month, 2 to 8 °C under sterile conditions after reconstitution.
- 2 months, -20 to -70°C under sterile conditions after reconstitution.

Activity Assay Protocol

Materials:

1. Assay Buffer: 50 mM MES, 50 mM NaCl, 5 mM DTT, pH 5.5
2. Recombinant Mouse Active Cathepsin C/DPPI (rmCathepsin C) (Catalog # 2336-CY)
3. Fluorogenic Peptide Substrate: Gly-Arg-AMC (Bachem, Cat # I-1215),10 mM stock in DMSO
4. F16 Black Maxisorp Plate (Nunc, Catalog # 475515)
5. Fluorescent Plate Reader (Model: SpectraMax Gemini EM by Molecular Devices) or equivalent

Assay:

1. Dilute rmCathepsin C to 0.02 ng/µL in Assay Buffer.
2. Dilute Substrate to 200 µM in Assay Buffer.
3. Load into a black well plate 50 µL of 0.02 ng/µL rmCathepsin C,and start the reaction by adding 50 µL of 200 µM Substrate. Include a Substrate Blank containing Assay Buffer in place of rmCat hepsin C.

4. Read at excitation and emission wavelengths of 380 nm and 460 nm (top read), respectively, in kin etic Mode for 5 minutes.
5. Calculate specific activity:

Specific Activity (pmol/min/µg) =
(Adjusted Vmax* (RFU/min) x Conversion Factor** (pmol/RFU))/amount of enzyme (µg)

*Adjusted for Substrate Blank

**Derived using calibration standard 7-Amino, 4-Methyl Coumarin (AMC) (Sigma, Catalog # A-9891).

Final Assay Conditions

Per Well:

1. rmCathepsin C: 0.001 µg
2. Substrate: 100 µM

3/15/2014

FOR RESEARCH USE ONLY.
NOT FOR USE IN HUMANS.