**DESCRIPTION**

**Source**
Mouse myeloma cell line, NS0-derived mouse Cathepsin C/DPPI protein proform (Asp25-Leu462 with a C-terminal 10-His tag)
Accession # P97821
The proform was activated by Recombinant Human Cathepsin L (Catalog # 952-CY) and further purified.

**N-terminal Sequence Analysis**
Asp25 (Exclusion domain) and Asp394 (Light chain)

**Structure / Form**
Active form

**Predicted Molecular Mass**
13 kDa (Exclusion domain), 18 kDa (Heavy chain), and 9 kDa (Light chain)

**SPECIFICATIONS**

<table>
<thead>
<tr>
<th>SDS-PAGE</th>
<th>8 kDa and 20-27 kDa, reducing conditions</th>
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</thead>
<tbody>
<tr>
<td>Activity</td>
<td>Measured by its ability to cleave the fluorogenic peptide substrate, Gly-Arg-7-amido-4-methylcoumarin (GR-AMC). The specific activity is &gt; 60,000 pmol/min/µg, as measured under the described conditions.</td>
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<tr>
<td>Endotoxin Level</td>
<td>&lt;1.0 EU per 1 µg of the protein by the LAL method.</td>
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<tr>
<td>Purity</td>
<td>&gt;95%, by SDS-PAGE under reducing conditions and visualized by silver stain.</td>
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<td>Formulation</td>
<td>Supplied as a 0.2 µm filtered solution in MES, NaCl and Glycerol. See Certificate of Analysis for details.</td>
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</table>

**Activity Assay Protocol**

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

**Assay**
1. Dilute rmCathepsin C to 0.01 ng/µL in Assay Buffer.
2. Dilute Substrate to 800 µM in Assay Buffer.
3. Load into a black well plate 50 µL of 0.01 ng/µL rmCathepsin C, and start the reaction by adding 50 µL of 800 µM Substrate. Include a Substrate Blank containing Assay Buffer in place of rmCathepsin C.
4. Read at excitation and emission wavelengths of 380 nm and 460 nm (top read), respectively, in kinetic mode for 5 minutes.
5. Calculate specific activity:

\[
\text{Specific Activity (pmol/min/µg)} = \frac{\text{Adjusted } V_{\text{max}}^* \cdot (\text{RFU/min}) \times \text{Conversion Factor}^{**}}{\text{amount of enzyme (µg)}}
\]

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

**Final Assay Conditions**
- rmCathepsin C: 0.0005 µg
- Substrate: 400 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.

**BACKGROUND**

Cathepsin C (CTSC) is a cysteine protease of the papain family (1). It sequentially removes dipeptides from the free N-termini of proteins and peptides. It has broad specificity except that it does not cleave a basic amino acid (Arg or Lys) in the N-terminal position or Pro on either side of the scissile bond. It requires halide ions for activity. The pro form contains a pro region and a mature region, which are further cleaved during activation to remove the prodomain and split the catalytic domain into heavy and light chains. The N-terminal domain of the pro region is also called the exclusion domain, which remains connected to the heavy chain through non-covalent bonds to the mature, active enzyme. Broadly distributed, CTSC plays a major role in lysosomal degradation and enzyme activation. For example, it activates granule serine proteases in cytotoxic T lymphocytes and natural killer cells (granzymes A and B), mast cells (tryptase and chymase), and neutrophils (Cathepsin G and elastase) by removing their N-terminal activation dipeptides (2).

**References:**