Recombinant Human Cathepsin H  
Catalog Number: 7516-CY

**DESCRIPTION**

<table>
<thead>
<tr>
<th>Source</th>
<th>Chinese Hamster Ovary cell line, CHO-derived human Cathepsin H protein Ala23-Val335, with a C-terminal 10-His tag. Accession # CAA34734</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-terminal Sequence Analysis</td>
<td>Ala23</td>
</tr>
<tr>
<td>Predicted Molecular Mass</td>
<td>37 kDa</td>
</tr>
</tbody>
</table>

**SPECIFICATIONS**

| SDS-PAGE | 38-43 kDa, reducing conditions |
| Activity | Measured by its ability to cleave the fluorogenic peptide substrate, Arg-7-amido-4-methylcoumarin (R-AMC). The specific activity is >750 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 | >80%, by SDS-PAGE visualized with Silver Staining and quantitative densitometry by Coomassie® Blue Staining. |
| 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: 50 mM MES, 10 mM CaCl<sub>2</sub>, 150 mM NaCl, pH 6.5
- Assay Buffer: 50 mM MES, pH 6.5
- Recombinant Human Cathepsin H (rhCathepsin H) (Catalog # 7516-CY)
- Bacterial Thermolysin (Thermolysin) (Catalog # 3097-ZN)
- Phosphoramidon (Tocris, Catalog # 6333), 20 mM stock in methanol
- Substrate: Arg-7-amino-4-methylcoumarin (R-AMC) (ChemImpex, Catalog # 5859), 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 rhCathepsin H to 200 µg/mL in Activation Buffer.
2. Dilute Thermolysin to 100 µg/mL in Activation Buffer.
3. Mix equal volumes of 100 µg/mL Thermolysin and 200 µg/mL rhCathepsin H.
4. Incubate at RT for 3 hours.
5. Stop reaction by adding an equal volume of 2 mM Phosphoramidon in Assay Buffer to reaction mixture.
6. Incubate at RT for 10 minutes.
7. Add an equal volume of Assay Buffer containing 20 mM DTT to reaction mixture. The concentration of rhCathepsin H is now 25 µg/mL.
8. Incubate reaction at RT for 5 minutes.
9. Dilute activated rhCathepsin H to 1 µg/mL in Assay Buffer.
10. Dilute Substrate to 200 µM in Assay Buffer.
11. In a plate, load 50 µL of 1 µg/mL rhCathepsin H to wells, and start the reaction by adding 50 µL of 200 µM Substrate. Include a Substrate Blank containing 50 µL Assay Buffer and 50 µL of 200 µM substrate.
12. Read at excitation and emission wavelengths of 380 nm and 460 nm (top read), respectively, in kinetic mode for 5 minutes.
13. Calculate specific activity:

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

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

**Final Assay Conditions**

Per Well:
- rhCathepsin H: 0.05 µg
- Substrate: 100 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, -70 °C as supplied.
- 3 months, -70 °C under sterile conditions after opening.
Cathepsin H is a lysosomal cysteine protease of the papain family (1). It is synthesized as a precursor protein that is proteolytically processed to a mature form consisting of a light chain, a heavy chain, and a mini-chain (2). Cathepsin H is the only known mono-aminopeptidase in the papain family (3). Cathepsin H is primarily an aminopeptidase, but also functions as an endopeptidase (4). Cathepsin H is potently inhibited by cystatins A, B, and C and by ovocystatin. Cathepsin H expression is altered in disease states such as prostate and colorectal cancers (5).

References: