

**DESCRIPTION**

**Source** *E. coli*-derived  
Lys2-Cys689, with an N-terminal Met and 6-His tag  
Accession # NP\_003240

**N-terminal Sequence Analysis** Met

**Predicted Molecular Mass** 80 kDa

**SPECIFICATIONS**

**SDS-PAGE** 80 kDa, reducing conditions

**Activity** Measured by its ability to cleave a fluorogenic peptide substrate, (7-methoxycoumarin-4-yl)acetyl-Pro-Leu-Gly-Pro-D-Lys(2,4-dinitrophenyl)-OH or Mca-PLGPK(Dnp)-OH.  
The specific activity is >250 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 MES, NaCl and Glycerol. See Certificate of Analysis for details.

**Activity Assay Protocol**

- Materials**
- Assay Buffer: 25 mM Tris, 150 mM NaCl, pH 7.5
  - Recombinant Human Thimet Oligopeptidase/THOP1 (rhTHOP1) (Catalog # 3439-ZN)
  - Substrate: MCA-Pro-Leu-Gly-Pro-D-Lys-(DNP)-OH (Bachem, Catalog # M-2270); 2 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 rhTHOP1 to 0.5 ng/μL in Assay Buffer.
  2. Dilute Substrate to 20 μM in Assay Buffer.
  3. Load 50 μL of the 0.5 ng/μL rhTHOP1 in 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.
  4. Read at excitation and emission wavelengths of 320 nm and 405 nm (top read), respectively, in kinetic mode for 5 minutes.
  5. 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:
- rhTHOP1: 0.025 μg
  - Substrate: 10 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**

Thimet Oligopeptidase/THOP1, also known as endopeptidase EC 3.4.24.15 (EP24.15), is a zinc peptidase of the M3 family that also includes neurolysin/EC 3.4.24.16 and mitochondrial intermediate peptidase (1). Widely expressed by mammalian tissues and reported to present in different subcellular locations, THOP1 is primarily a cytoplasmic enzyme. It is capable of hydrolyzing a number of bioactive peptides and peptides released by the proteasome, limiting antigenic presentation by MHC class I molecules (1-3). THOP1 also interacts with angiotensin II type I receptor and bradykinin B2 receptor (4). The optimal activity of the purified THOP1 may or may not require the presence of a reducing agent, depending upon the source of the enzyme and the purification method used.

**References:**

1. Barrett, A.J. and J.-M. Chen (2004) in *Handbook of Proteolytic Enzymes*. Barrett, A.J. *et al.* eds. p. 352, Elsevier Academic Press, San Diego.
2. Ray, K. *et al.* (2004) *J. Biol. Chem.* **279**:20480.
3. Saric, T. *et al.* (2004) *J. Biol. Chem.* **279**:46723.
4. Shivakumar, B.R. *et al.* (2005) *Cell Biochem. Funct.* **23**:195.