

DESCRIPTION

Source Mouse myeloma cell line, NS0-derived
Met1-Ala468, with a C-terminal 10-His tag
Accession # Q61398

N-terminal Sequence Analysis No result, Gln25 predicted.

Predicted Molecular Mass 49 kDa

SPECIFICATIONS

SDS-PAGE 52-57 kDa, reducing conditions

Activity Measured by the stimulation of procollagen type I processing by BMP-1.
>50% of the full-length procollagen, in the presence of recombinant mouse PCPE, is processed by BMP-1, as measured under the described conditions.

Endotoxin Level <1.0 EU per 1 µg of the protein by the LAL method.

Purity >90%, by SDS-PAGE under reducing conditions and visualized by Colloidal Coomassie® Blue stain at 5 µg per lane.

Formulation Supplied as a 0.2 µm filtered solution in MES and NaCl. See Certificate of Analysis for details.

Activity Assay Protocol

- Materials**
- Dilution Buffer: 50 mM Tris, 5 mM CaCl₂, pH 7.5
 - Assay Buffer: 50 mM Tris, 5 mM CaCl₂, 150 mM NaCl, pH 7.5 (TCN)
 - Recombinant Mouse PCPE-1 (rmPCPE-1) (Catalog # 2239-PE)
 - Recombinant Human Pro-Collagen I α1 (rhPro-COL1A1) (Catalog # 6220-CL)
 - Recombinant Human BMP-1/PCP (rhBMP-1) (Catalog # 1927-ZN)
 - Reducing SDS-PAGE Sample Buffer
 - SDS-PAGE or Western Blot

- Assay**
1. Dilute rhPro-COL1A1 to 75 µg/mL in Dilution Buffer.
 2. Dilute rmPCPE-1 to 15 µg/mL in Assay Buffer.
 3. Combine 20 µL of diluted rhPro-COL1A1 and 20 µL of diluted rmPCPE-1. Prepare a rhPro-COL1A1 control by combining 20 µL of rhPro-COL1A1 and 20 µL of Assay Buffer. Prepare one rmPCPE-1 control by combining 20 µL of rmPCPE-1 and 20 µL of Assay Buffer (optional).
 4. Incubate reaction vials and controls at 37 °C for 30 minutes.
 5. Dilute rhBMP-1 to 1.875 µg/mL in Assay Buffer.
 6. Add 20 µL of diluted rhBMP-1 to each reaction vial, excluding the rhPro-COL1A1 control. Add 20 µL of Assay Buffer to the rhPro-COL1A1 control in place of rhBMP-1.
 7. Incubate reaction vials and controls at 37 °C for 30 minutes.
 8. After incubation, combine 30 µL of each reaction mixture and control with 30 µL of reducing SDS-PAGE gel buffer. Mix and incubate samples at 95-100 °C for 3-5 minutes to stop reactions.
 9. Load 40 µL (0.5 µg of rhPro-COL1A1) per lane and analyze the cleavage by SDS-PAGE followed by protein staining and/or Western blot.
 10. Activity calculation:

$$\% \text{ Cleavage} = \left[1 - \frac{\% \text{ full-length rhPro-COL1A1 (reaction)}}{\% \text{ full-length rhPro-COL1A1 (control)}} \right] \times 100\%$$

- Final Assay Conditions**
- Per Lane
- rmPCPE-1: 0.1 µg
 - rhPro-COL1A1: 0.5 µg
 - rhBMP-1: 0.0125 µg

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

Procollagen C-endopeptidase enhancers, known as PCPEs or PCOLCEs, are secreted extracellular matrix glycoproteins that consist of two CUB domains and one NTR domain. They are known to stimulate enzymatic cleavage of procollagens I-III by the BMP-1/tolloid family of metalloproteases, also known as procollagen C-proteinases (1). PCPE-1 is expressed primarily by interstitial connective tissues such as tendons, calvaria, and skin (2). Although BMP-1/tolloid proteinases are involved in processing of multiple extracellular proteins, the enhancer activity of PCPE-1 is specific to procollagens since it has no effect on BMP-1/tolloid cleavage of other substrates (3). It is thought that PCPE-1 enhances cleavage of procollagens by binding to the substrate, inducing a conformation change in the substrate (3), although interaction between PCPE-1 and full-length BMP-1/tolloid proteinases has also been reported (4).

References:

1. Steiglitz, B.M. *et al.* (2002) *J. Biol. Chem.* **277**:49820.
2. Kessler, E. *et al.* (1990) *Biochem. Biophys. Res. Commun.* **173**:81.
3. Moali, C. *et al.* (2005) *J. Biol. Chem.* **280**:24188.
4. Ge, G. *et al.* (2006) *J. Biol. Chem.* **281**:10786.

PRODUCT SPECIFIC NOTICES

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