Recombinant Human BMP-1/PCP

Catalog Number: 1927-ZN

DESCRIPTION

Source	Mouse myeloma cell line, NS0-derived Ala121-Gln730, with a C-terminal 6-His tag Accession # NP_001190
N-terminal Sequence Analysis	Ala121
Predicted Molecular Mass	70.5 kDa

SPECIFICATIONS

SDS-PAGE	84 kDa, reducing conditions
Activity	Measured by its ability to cleave a fluorogenic peptide substrate, Mca-YVADAPK(Dnp)-OH (Catalog # ES007). The specific activity is >4 pmol/min/µg, as measured under the described conditions. See Activity Assay Protocol on www.RnDSystems.com
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	Lyophilized from a 0.2 µm filtered solution in HEPES, Sodium Sulfate and $ZnCl_2$. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 25 mM HEPES, 0.1% Brij-35 (w/v), pH 7.5
- Recombinant Human BMP−1/PCP (rhBMP-1) (Catalog # 1927-ZN)
- Fluorogenic Peptide Substrate: MCA-Tyr-Val-Ala-Asp-Ala-Pro-Lys(DNP)-OH (Catalog # ES007)
- F16 Black Maxisorp Plate (Nunc, Catalog # 475515)
- Fluorescent Plate Reader (Model: SpectraMax Gemini EM by Molecular Devices) or equivalent

Assay

1. Dilute rhBMP-1 to 20 ng/µL in Assay Buffer.
2. Dilute substrate to 20 µM in Assay Buffer.
3. Load into a black well plate 50 µL of 20 ng/µL rhBMP-1 and start the reaction by adding 50 µL of 20 µM Substrate. Include a Substrate Blank containing Assay Buffer in place of rhBMP-1.
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:

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

*Adjusted for Substrate Blank

**Derived using calibration standard MCA-Pro-Leu-OH (Bachem, Catalog # M-1975).

Final Assay Conditions

Per Well:

- rhBMP-1: 1 µg
- Substrate: 10 µM

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 100 µg/mL in sterile 25 mM HEPES and 0.01% (w/v) Brij-35, pH 7.5.
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.** • 6 months from date of receipt, -20 to -70 °C as supplied. • 3 months, -20 to -70 °C under sterile conditions after reconstitution.

BACKGROUND

Bone morphogenetic protein 1 (BMP-1), also known as procollagen C-proteinase (PCP), is a zinc protease of the astacin family (1, 2). BMP-1/PCP plays a key role in formation of extracellular matrix (ECM) by converting precursor proteins into their mature and functional forms. The precursor proteins identified as substrates for BMP-1/PCP include collagens, biglycan, laminin 5, dentin matrix protein-1, and lysyl oxidase (3). There are six alternatively spliced forms known to be derived from the BMP-1 gene, and isoform 1 consisting of residues 1 to 730 was expressed. The secreted and purified protein does not contain the signal peptide (amino acid residues 1-22) and pro domain (residues 23-120), but contain protease (residues 121-321), CUB I (residues 322-434), CUB II (residues 435-546), EGF-like (residues 547-588) and CUB III (residues 591-703) domains. The pro domain is apparently cleaved by a furin-like proprotein convertase (4). The purified BMP-1/PCP is an active protease and its peptidase activity can be determined as described above. The purified BMP-1/PCP is predicted to possess procollagen C-proteinase activity because it contains the minimal domain structure required (5).

References:

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2. Bond, J.S. and R.J. Beynon (1995) Protein Sci. **4**:1247.
3. Steiglitz, B.M. *et al.* (2004) J. Biol. Chem. **279**:980.
4. Leighton, M. and K.E. Kadler (2003) J. Biol. Chem. **278**:18478.
5. Hartigan, N. *et al.* (2003) J. Biol. Chem. **278**:18045.

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