

**DESCRIPTION**

|                                     |  |
|-------------------------------------|--|
| <b>Source</b>                       | Mouse myeloma cell line, NS0-derived human BMP-4 protein<br>Ser293-Arg408<br>Accession # P12644.1<br>Manufactured and tested under current Good Manufacturing Practice (GMP) guidelines. |
| <b>N-terminal Sequence Analysis</b> | Ser <sup>293</sup> -Pro-Lys-His-His-Ser-Gln-Arg-Ala-Arg  |
| <b>Structure / Form</b>             | Disulfide-linked homodimer   |
| <b>Predicted Molecular Mass</b>     | 13 kDa (monomer)   |

**SPECIFICATIONS**

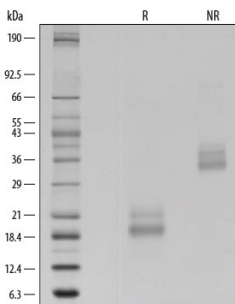
|                           |   |
|---------------------------|---|
| <b>SDS-PAGE</b>           | 22-25 kDa, reducing conditions<br>37-41 kDa, non-reducing conditions  |
| <b>Activity</b>           | Measured by its ability to induce alkaline phosphatase production by ATDC5 mouse chondrogenic cells. Binnerts, M.E. <i>et al.</i> (2004) <i>Biochem. Biophys. Res. Commun.</i> <b>315</b> (2):272.<br>The ED <sub>50</sub> for this effect is 2.5-15 ng/mL. |
| <b>Endotoxin Level</b>    | <0.01 EU per 1 µg of the protein by the LAL method.   |
| <b>Purity</b>             | >95%, by SDS-PAGE with silver staining, under reducing conditions.  |
| <b>Host Cell Protein</b>  | < 5.0 ng per µg of protein when tested by ELISA.  |
| <b>Mycoplasma</b>         | Negative when tested in a ribosomal RNA hybridization assay.  |
| <b>Adventitious Virus</b> | Master Cell Bank tested for adventitious viruses  |
| <b>Formulation</b>        | Lyophilized from a 0.2 µm filtered solution in Acetonitrile and TFA. See Certificate of Analysis for details.   |

**PREPARATION AND STORAGE**

|                                |  |
|--------------------------------|--|
| <b>Reconstitution</b>          | Reconstitute at 50-200 µg/mL in 4 mM HCl.  |
| <b>Shipping</b>                | The product is shipped with polar packs. Upon receipt, store it immediately at the temperature recommended below.  |
| <b>Stability &amp; Storage</b> | <b>Use a manual defrost freezer and avoid repeated freeze-thaw cycles.</b> <ul style="list-style-type: none"> <li>• A minimum of 6 months when stored at ≤ -20 °C as supplied. Refer to lot specific COA for the Use by Date.</li> <li>• 1 month, 2 to 8 °C under sterile conditions after reconstitution.</li> <li>• 3 months, -70 °C under sterile conditions after reconstitution.</li> </ul> |

**DATA**

**SDS-PAGE**



**Recombinant Human BMP-4 GMP Protein SDS-PAGE** 1 µg/lane of GMP-grade Recombinant Human BMP-4 (Catalog # 314-GMP) was resolved with SDS-PAGE under reducing (R) and non-reducing (NR) conditions and visualized by silver staining, showing bands at 19 and 21 kDa, and 35 and 38 kDa, respectively.

## BACKGROUND

BMP-4 is a TGF- $\beta$  superfamily ligand that is widely expressed from early embryogenesis through adulthood. It plays an important role in mesenchyme formation, epidermal determination, suppression of neural induction, the development of multiple organs, and tissue repair (1-5). The human BMP-4 precursor contains a 273 amino acid (aa) propeptide and a 116 aa mature protein (6). Processing of the propeptide by furin or proprotein convertase 6 enables the formation of the mature disulfide-linked homodimeric BMP-4 and facilitates its secretion. Similar intracellular processes may lead to the formation and recreation of BMP4/BMP7 disulfide-linked heterodimer (7-9). Mature human and mouse BMP-4 share 98% aa sequence identity. Human BMP-4 shares 85% aa sequence identity with human BMP-2 and less than 50% with other human BMPs. Compared to BMP-4 homodimers, BMP-4/BMP-7 heterodimers exhibit a greater potency in inducing osteogenic differentiation (9). In *Xenopus*, the heterodimers can also induce the formation of mesoderm, whereas BMP-4 homodimers only provide ventralizing signals for existing mesoderm (10). BMP-4 signals through tetrameric complexes composed of type I (primarily Activin RIA or BMPRI-A) and type II (primarily Activin RIIA or BMPRII) receptors (11, 12). The bioavailability of BMP-4 is regulated by its interaction with multiple proteins and glycosaminoglycans (13-15).

## References:

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6. Wozney, J. *et al.* (1988) *Science* **242**:1528.
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8. Cui, Y. *et al.* (2001) *Genes Dev.* **15**:2797.
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12. Lavery, K. *et al.* (2008) *J. Biol. Chem.* April 24 epub.
13. Rosen, V. (2006) *Ann. N.Y. Acad. Sci.* **1068**:19.
14. Jones, C.M. and J.C. Smith (1998) *Dev. Biol.* **194**:12.
15. Takada, T. *et al.* (2003) *J. Biol. Chem.* **278**:43229.

## MANUFACTURING SPECIFICATIONS

### GMP Proteins

R&D Systems, a Bio-Techne Brand's GMP proteins are produced according to relevant sections of the following documents: WHO TRS, No. 822, 1992 Annex 1, Good Manufacturing Practices for Biological Products; USP Chapter 1043, Ancillary Materials for Cell, Gene and Tissue-Engineered Products and USP Chapter 92, Growth Factors and Cytokines Used in Cell Therapy Manufacturing.

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- Monitoring of stability over product shelf-life

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- Post-bottling lot-specific bioassay results (compliance with an established range) and results of microbial testing according to USP
- Host Cell Protein testing performed by ELISA
- Mycoplasma testing by ribosomal RNA hybridization assay

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