

DESCRIPTION

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

SPECIFICATIONS

SDS-PAGE	22-25 kDa, reducing conditions 37-41 kDa, non-reducing conditions
Activity	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> 315(2) :272. The ED ₅₀ for this effect is 2.5-15 ng/mL.
Endotoxin Level	<0.01 EU per 1 µg of the protein by the LAL method.
Purity	>95%, by SDS-PAGE with silver staining, under reducing conditions.
Host Cell Protein	< 5.0 ng per µg of protein when tested by ELISA.
Mycoplasma	Negative when tested in a ribosomal RNA hybridization assay.
Adventitious Virus	Master Cell Bank tested for adventitious viruses
Formulation	Lyophilized from a 0.2 µm filtered solution in Acetonitrile and TFA. See Certificate of Analysis for details.

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 50-200 µg/mL in 4 mM HCl.
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. <ul style="list-style-type: none"> A minimum of 6 months when stored at ≤ -20 °C as supplied. Refer to lot specific COA for the Use by Date. 1 month, 2 to 8 °C under sterile conditions after reconstitution. 3 months, -70 °C under sterile conditions after reconstitution.

DATA

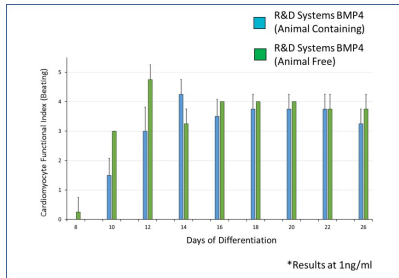
Cell Differentiation/Maturation

Animal-Free Recombinant Human BMP-4 GMP Protein induces mesoderm differentiation Mesoderm differentiation was evaluated by fold increase of nuclear brachyury with Animal-Free Catalog # **314-GMP** showing the highest differentiation.

Cell Differentiation/Maturation

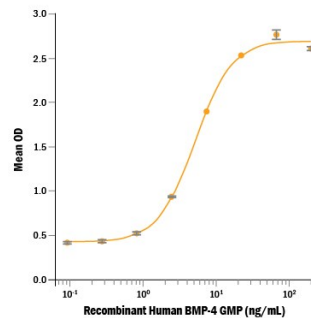
Animal-Free GMP-4 exhibits full functionality Chondrocyte differentiation assay with animal-free recombinant human BMP-4 GMP protein (Cat # Catalog # **314-GMP**) exhibits similar differentiation profile as hBMP-4 synthesized in NS0 cell cultures.

Cell Differentiation/Maturation



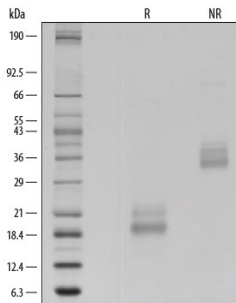
Cardiac Differentiation Function in vitro Scoring System to determine cardiac function in vitro (Beating) for animal-containing BMP-4 and Animal-Free Catalog # 314-GMP: 0 = No beating in entire dish 1 = Very Sparse, isolated beating. Individual Cells and small clusters. 2 = Small clusters of beating cells in most (>50%) of fields 3 = Widespread beating in all fields with some limited coordination and rhythmic beating 4 = Most of the dish (>75%) exhibits coordinated beating and rhythmic beating. May be able to see it by eye, but with difficulty 5 = Full dish exhibits strong, coordinated and rhythmic beating. Can easily see it by eye without the aid of a microscope

Bioactivity



Recombinant Human BMP-4 GMP Protein Bioactivity GMP-grade Recombinant Human BMP-4 (Catalog # 314-GMP) induces alkaline phosphatase production in the ATDC5 mouse chondrogenic cell line. The ED₅₀ for this effect is 2.5-15 ng/mL.

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-β 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 BMPR-IA) and type II (primarily Activin RIIA or BMPR-II) 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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2. Gambaro, K. *et al.* (2006) *Cell Death Differ.* **13**:1075.
3. Simic, P. and S. Vukicevic (2005) *Cytokine Growth Factor Rev.* **16**:299.
4. Sadlon, T.J. *et al.* (2004) *Stem Cells* **22**:457.
5. Frank, D.B. *et al.* (2005) *Circ. Res.* **97**:496.
6. Wozney, J. *et al.* (1988) *Science* **242**:1528.
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9. Aono, A. *et al.* (1995) *Biochem. Biophys. Res. Commun.* **210**:670.
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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

R&D Systems strives to provide our customers with the analytical characteristics of each product so that customers may determine whether our products are appropriate for their research. The Certificate of Analysis provided contains the following lot specific information:

- N-terminal amino acid analysis, SDS-PAGE analysis, and endotoxin level (as determined by LAL assay) performed on each bulk QC lot, not on individual bottlings of each QC lot
- 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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