Recombinant Human Wnt-3a GMP

Catalog Number: 5036-GMP

DESCRIPTION

Source	Chinese Hamster Ovary cell line, CHO-derived human Wnt-3a protein Ser19-Lys352 Accession # NP_149122 Manufactured and tested under cGMP guidelines.
N-terminal Sequence Analysis	Ser_{19}-Tyr-Pro-Ile-Trp-Trp-Ser-Leu-Ala-Val
Predicted Molecular Mass	37.4 kDa

SPECIFICATIONS

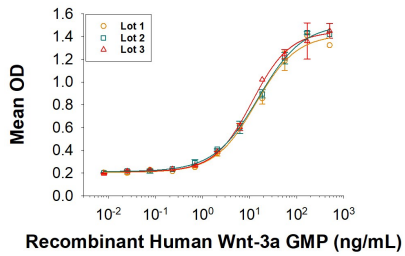
SDS-PAGE	40 kDa, reducing conditions
Activity	Measured by its ability to induce alkaline phosphatase production by MC3T3-E1 mouse preosteoblast cells. The ED_{50} for this effect is 5-25 ng/mL. Measured by its ability to induce Topflash reporter activity in HEK293T human embryonic kidney cells. The ED_{50} for this effect is <500 ng/mL. Protein concentrations should be titrated based on cell type and if appropriate, passage number of the cell line. **Optimal concentrations should be determined by each laboratory for each application.**
Endotoxin Level	<0.10 EU per 1 µg of the protein by the LAL method.
Purity	>75%, by SDS-PAGE with silver staining, under reducing conditions.
Host Cell Protein	< 40 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 PBS, EDTA and CHAPS. See Certificate of Analysis for details.

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 200 µg/mL in PBS.
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.** • 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, ≤ -20 °C under sterile conditions after reconstitution.

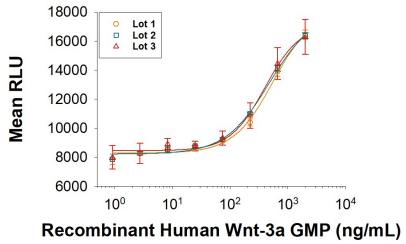
DATA

Bioactivity



GMP-grade Recombinant Human Wnt-3a (Catalog # 5036-GMP) induces alkaline phosphatase production by the MC3T3-E1 mouse preosteoblast cell line. The ED_{50} for this effect is 5-25 ng/mL. Three independent lots were tested for activity and plotted on the same graph to show lot-to-lot consistency of GMP Wnt-3a.

Bioactivity



GMP-grade Recombinant Human Wnt-3a (Catalog # 5036-GMP) induces β-catenin-responsive transcriptional activation in a Topflash Luciferase assay using the HEK293T human embryonic kidney cell line. The ED_{50} for this effect is <500 ng/mL. Three independent lots were tested for activity and plotted on the same graph to show lot-to-lot consistency of GMP Wnt-3a.

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BACKGROUND

Wnt-3a is one of 19 vertebrate members of the Wingless-type MMTV integration site (Wnt) family of highly conserved cysteine-rich secreted glycoproteins important for normal developmental processes (1). Wnts bind to the cell surface Frizzled family receptors in conjunction with low-density lipoprotein receptor-related protein family receptors (LRP5 or 6) resulting in the stabilization of intracellular β-catenin levels (2). As intracellular β-catenin levels rise, β-catenin binds to TCF/LEF transcription factors leading to expression of Wnt target genes (3). Endo-IWR 1 (Catalog # 3532, # PSM1324) is a cell-permeant small molecule inhibitor of Axin turnover that suppresses Wnt signal transduction by stabilizing the β-catenin destruction complex (4). Wnt-3a is a 44 kDa secreted hydrophobic glycoprotein containing a conserved pattern of 24 cysteine residues (5). Wnt-3a has two N-linked glycosylation sites (Asn 87, Asn 298), and Ser 209 is modified with palmitoleic acid (6). Glycosylation and acylation are essential for efficient Wnt secretion and biological activity, respectively (6, 7). Human Wnt-3a shares 96% amino acid (aa) identity with mouse mouse, bovine and canine Wnt-3a, and 89%, 86% and 84% aa identity with chicken, Xenopus and zebrafish Wnt-3a, respectively. It also shares 87% aa identity with Wnt3. During embryonic development, Wnt-3a is necessary for proper development of the hippocampus, anterior-posterior patterning, somite development, and tailbud formation (9-12). Wnt-3a also promotes self-renewal of hematopoietic stem cells, neural stem cells, and embryonic stem cells (13, 14).

References:

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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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