

DESCRIPTION

Source Chinese Hamster Ovary cell line, CHO-derived human Wnt-3a protein
Ser19-Lys352
Accession # NP_149122.1
Manufactured and tested under cGMP guidelines.

N-terminal Sequence Analysis Ser¹⁹-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 ED₅₀ for this effect is 5-25 ng/mL.

Measured by its ability to induce Topflash reporter activity in HEK293T human embryonic kidney cells.
The ED₅₀ 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 for Mycoplasma.

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.

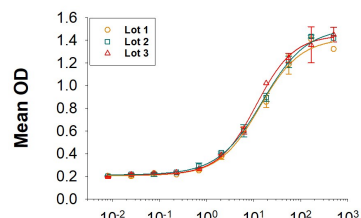
Shipping The product is shipped with polar packs. Upon receipt, store it immediately at the temperature recommended below.

Stability & Storage Use a manual defrost freezer and avoid repeated freeze-thaw cycles.

- A minimum of 12 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

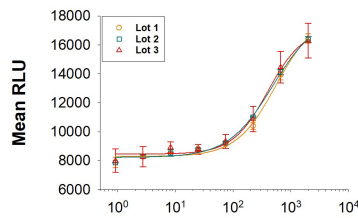
Bioactivity



Recombinant Human Wnt-3a GMP (ng/mL)

Recombinant Human Wnt-3a GMP Protein Bioactivity GMP-grade Recombinant Human Wnt-3a (Catalog # 5036-GMP) induces alkaline phosphatase production by the MC3T3-E1 mouse preosteoblast cell line. The ED₅₀ 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



Recombinant Human Wnt-3a GMP (ng/mL)

Recombinant Human Wnt-3a GMP Protein 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 ED₅₀ 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.

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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- Willert, K. *et al.* (2003) Nature **423**:6938.
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- Ten Berge, D. *et al.* (2011) Nat. Cell Biol. **13**:1070.

MANUFACTURING SPECIFICATIONS

GMP Proteins

R&D Systems, a Bio-Techne Brand's GMP proteins are produced according to relevant sections of the following documents: USP Chapter 1043, Ancillary Materials for Cell, Gene and Tissue-Engineered Products and Eu. Ph. 5.2.12, Raw Materials of Biological Origin for the Production of Cell-based and Gene Therapy Medicinal Products.

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- Fully validated equipment, processes and test methods
- Equipment calibration schedules using a computerized calibration program
- Facility maintenance, safety programs and pest control
- Material review process for variances
- 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:

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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

Additional testing and documentation requested by the customer can be arranged at an additional cost.

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