

DESCRIPTION

Source *Spodoptera frugiperda*, Sf 21 (baculovirus)-derived human Dkk-1 protein
Thr32-His266
Accession # O94907
Manufactured and tested under cGMP guidelines.

N-terminal Sequence Analysis Thr-Leu-Asn-Ser-Val-Leu-Asn-Ser-Asn-Ala

Predicted Molecular Mass 25.8 kDa

SPECIFICATIONS

SDS-PAGE 33-38 kDa, reducing conditions

Activity Measured by its ability to inhibit Wnt induced TCF reporter activity in HEK293 human embryonic kidney cells. Recombinant Human Dkk-1 (Catalog # 5439-GMP) inhibits a constant dose of 500 ng/mL of Recombinant Human Wnt-3a (Catalog # 5036-WN). The ED₅₀ for this effect is 10-60 ng/mL.

Endotoxin Level <1.0 EU per 1 µg of the protein by the LAL method.

Purity >95%, by SDS-PAGE with silver staining.

Mycoplasma Negative when tested in a ribosomal RNA hybridization assay.

Formulation Lyophilized from a 0.2 µm filtered solution in PBS. See Certificate of Analysis for details.

PREPARATION AND STORAGE

Reconstitution Reconstitute at 100 µg/mL in PBS.

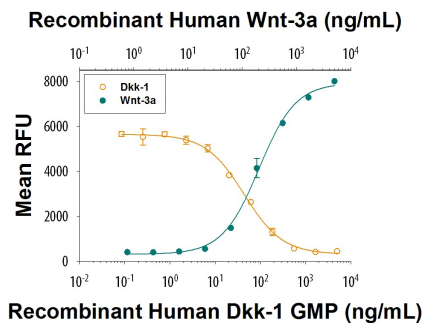
Shipping The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below.

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

- 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.

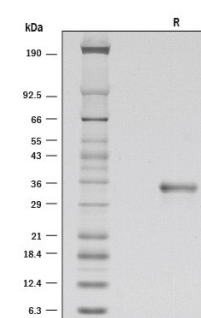
DATA

Bioactivity



Recombinant Human Wnt-3a (Catalog # 5036-WN) induces a dose responsive increase in Wnt reporter activity in HEK293 cells (green circles). Recombinant Human Dkk-1 GMP inhibits a constant dose of 500 ng/mL of Recombinant Human Wnt-3a. The ED₅₀ for this effect is 10-60 ng/mL (orange circles).

SDS-PAGE



1 µg/lane of Recombinant Human GMP-grade Dkk-1 was resolved with SDS-PAGE under reducing (R) conditions and visualized by silver staining, showing a band at 34 kDa.

BACKGROUND

Dickkopf related protein 1 (Dkk-1) is the founding member of the Dickkopf family of proteins that includes Dkk-1, -2, -3, -4, and a related protein, Soggy (1, 2). Dkk proteins are secreted proteins that contain two conserved cysteine-rich domains separated by a linker region. Each domain contains ten cysteine residues (1-3). Mature human Dkk-1 is a 40 kDa glycosylated protein that shares 86%, 87%, 90% and 91% aa sequence identity with mouse, rat, rabbit and bovine Dkk-1, respectively. It also shares 42% and 36% aa identity with human Dkk-2 and Dkk-4, respectively. Dkk-1 and Dkk-4 are well documented antagonists of the canonical Wnt signaling pathway (1, 2). This pathway is activated by Wnt engagement of a receptor complex composed of the Frizzled proteins and one of two low-density lipoprotein receptor-related proteins, LRP5 or LRP6 (4). Dkk-1 antagonizes Wnt by forming ternary complexes of LRP5/6 with Kremen1 or Kremen2 (4, 5). Dkk-1/LRP6/Krm2 complex internalization has been shown to downregulate Wnt signaling (4, 5). Dkk-1 is expressed throughout development and antagonizes Wnt-7a during limb development (6, 7). Other sites of expression include developing neurons, hair follicles and the retina of the eye (8, 9). The balance between Wnt signaling and Dkk-1 inhibition is critical for bone formation and homeostasis (10). Insufficient or excess Dkk-1 activity in bone results in increased or decreased bone density, respectively (8, 11). In adults, Dkk-1 is expressed in osteoblasts and osteocytes, and neurons. Cerebral ischemia induces Dkk-1 expression, which contributes to neuronal cell death (12).

References:

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2. Niehrs, C. (2006) *Oncogene* **25**:7469.
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4. Mao, B. *et al.* (2001) *Nature* **411**:321.
5. Mao, B. *et al.* (2002) *Nature* **417**:664.
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7. Adamska, M. *et al.* (2004) *Dev. Biol.* **272**:134.
8. Li, J. *et al.* (2006) *Bone* **36**:754.
9. Verani, R. *et al.* (2006) *J. Neurochem.* **101**:242.
10. Pinzone, J.J. *et al.* (2009) *Blood* **113**:517.
11. Morvan, F. *et al.* (2006) *J. Bone Miner. Res.* **21**:934.
12. Cappuccio, I. *et al.* (2005) *J. Neurosci.* **25**:2647.

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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- Personnel training programs
- Raw material testing and vendor qualification/monitoring
- 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

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- Post-bottling lot-specific bioassay results (compliance with an established range) and results of microbial bioburden testing (using broth culture, Sabourand's dextrose and blood agar plates with results reported at 3 days and at 7 days)
- Mycoplasma testing by ribosomal RNA hybridization assay

Additional testing and documentation requested by the customer can be arranged at an additional cost. Testing may include, but is not limited to, USP <61> bioburden testing, positive identity testing, testing for adventitious agents and testing for residual host cell content.

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