

DESCRIPTION

Source Mouse myeloma cell line, NS0-derived mouse Dkk-4 protein
Leu19-Ile221 (Arg67Ser and Arg70Ser), with a C-terminal 6-His tag
Accession # Q8VEJ3

N-terminal Sequence Analysis Leu19

Predicted Molecular Mass 23 kDa

SPECIFICATIONS

SDS-PAGE 31 kDa, reducing conditions

Activity Measured by its ability to inhibit proliferation in MC3T3-E1 mouse preosteoblast cells.
The ED₅₀ for this effect is 1-4 µg/mL.
Optimal concentrations should be determined by each laboratory for each application.

Measured by its ability to inhibit Wnt induced TCF reporter activity in HEK293 human embryonic kidney cells.
Recombinant Mouse Dkk-4 (Catalog # 3105-DK/CF) inhibits a constant dose of 100 ng/mL of Recombinant Mouse Wnt-3a (Catalog # 1324-WN). The ED₅₀ for this effect is 0.2-1.2 µg/mL.

Endotoxin Level <0.10 EU per 1 µg of the protein by the LAL method.

Purity >95%, by SDS-PAGE under reducing conditions and visualized by silver stain.

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 sterile PBS.

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

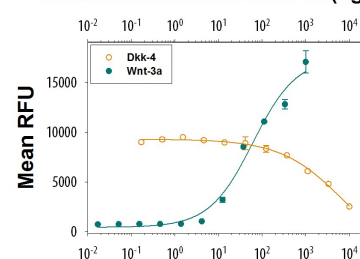
Stability & Storage Use a manual defrost freezer and avoid repeated freeze-thaw cycles.

- 12 months from date of receipt, -20 to -70 °C as supplied.
- 1 month, 2 to 8 °C under sterile conditions after reconstitution.
- 3 months, -20 to -70 °C under sterile conditions after reconstitution.

DATA

Binding Activity

Recombinant Mouse Wnt-3a (ng/mL)



Recombinant Mouse Dkk-4 Protein Binding Activity

Recombinant Mouse Wnt-3a (Catalog # 1324-WN) induces a dose responsive increase in Wnt reporter activity in HEK293 cells (green circles). Recombinant Mouse Dkk-4 (Catalog # 3105-DK/CF) inhibits a constant dose of 100 ng/mL of Recombinant Mouse Wnt-3a. The ED₅₀ for this effect is 0.2-1.2 µg/mL (orange circles).

Recombinant Mouse Dkk-4 (ng/mL)

BACKGROUND

Dickkopf related protein 4 (Dkk-4) is a member of the Dickkopf protein family that includes Dkk-1, -2, -3, and -4 and a related protein, Soggy (1). Expression of Dkk-4 has only been shown with sensitive PCR techniques during early embryonic development in mice (2), in differentiated human ES cells (3), or in mice that express dominant-active β -catenin elevating Wnt signaling in the forebrain (4). Dkk proteins are secreted proteins that are synthesized as precursors with an N-terminal signal peptide; all have two conserved cysteine-rich domains separated by a linker region which contains a potential furin type proteolytic cleavage site. The domains contain 10 cysteines each; prokineticin and colipase families show a configuration of cysteines similar to the second motif (5). Mouse Dkk-4 shows 91%, 72% and 71% amino acid (aa) identity with rat, human and canine Dkk-4, respectively, and 40-46% aa identity with other mouse Dkk proteins. Dkk-4 is predicted as a 25 kDa protein, but transfection of 293T cells produces a shorter (15-17 kDa) form containing only the second cysteine-rich domain, as well as longer (30-32 and 40 kDa) forms that do not appear to be glycosylated or form covalent multimers (1). Of the four Dkk proteins, Dkk-4 is the most like Dkk-1. Both are unequivocal antagonists of the canonical Wnt signaling pathway (1, 6), which is activated by Wnt protein engagement of a receptor complex composed of the Frizzled proteins and one of two low-density lipoprotein receptor-related proteins, LRP5 or LRP6 (7). Dkk-1 and Dkk-4 antagonize Wnt by direct high-affinity binding to LRP5/6, forming ternary complexes of LRP5/6 with the Kremens protein Krm2. Internalization of the complex is triggered, making LRP5/6 unavailable for interaction with Wnt ligands (6-9).

References:

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4. Diep, D.B. *et al.* (2004) *Dev. Brain Res.* **153**:261.
5. Bullock, C.M. *et al.* (2004) *Mol. Pharmacol.* **65**:582.
6. Mao, B. and C. Niehrs (2003) *Gene* **302**:179.
7. Logan, C.Y. and R. Nusse (2004) *Ann. Rev. Cell Dev. Biol.* **20**:781.
8. Mao, B. *et al.* (2002) *Nature* **417**:664.
9. Mao, B. *et al.* (2001) *Nature* **411**:321.