

Dkk-2 siRNA (h): sc-37084

BACKGROUND

The Wnt genes are a group of well conserved, cysteine-rich secreted glycoproteins that are required for numerous developmental processes including embryogenesis, asymmetric cell division and central nervous system (CNS) patterning. Wnt association with the seven membrane spanning receptor Frizzled, activates Dishevelled, which downregulates glycogen synthase kinase (GSK) through serine phosphorylation, causing the accumulation of β -catenin and subsequent regulation of developmentally significant Wnt target genes. The Dickkopf family of secreted inhibitors of Wnt signaling ensures proper morphological development by antagonizing different stages of the Wnt cascade. Dkk-2 (Dickkopf-2) is a 259-amino acid secreted protein that is composed of an N-terminal signal peptide and two conserved cysteine-rich domains, which are separated by a 50-55-amino acid linker region.

REFERENCES

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2. Cadigan, K.M. and Nusse, R. 1997. Wnt signaling: a common theme in animal development. *Genes Dev.* 11: 3286-3305.
3. Sakanaka, C., et al. 1998. Bridging of β -catenin and glycogen synthase kinase-3 β by axin and inhibition of β -catenin-mediated transcription. *Proc. Natl. Acad. Sci. USA* 95: 3020-3023.
4. Glinka, A., et al. 1998. Dickkopf-1 is a member of a new family of secreted proteins and functions in head induction. *Nature* 391: 357-362.
5. Fedi, P., et al. 1999. Isolation and biochemical characterization of the human Dkk-1 homologue, a novel inhibitor of mammalian Wnt signaling. *J. Biol. Chem.* 274: 19465-19472.
6. Krupnik, V.E., et al. 1999. Functional and structural diversity of the human Dickkopf gene family. *Gene* 238: 301-313.
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CHROMOSOMAL LOCATION

Genetic locus: DKK2 (human) mapping to 4q25.

PRODUCT

Dkk-2 siRNA (h) is a pool of 3 target-specific 19-25 nt siRNAs designed to knock down gene expression. Each vial contains 3.3 nmol of lyophilized siRNA, sufficient for a 10 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Dkk-2 shRNA Plasmid (h): sc-37084-SH and Dkk-2 shRNA (h) Lentiviral Particles: sc-37084-V as alternate gene silencing products.

For independent verification of Dkk-2 (h) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-37084A, sc-37084B and sc-37084C.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

STORAGE AND RESUSPENSION

Store lyophilized siRNA duplex at -20° C with desiccant. Stable for at least one year from the date of shipment. Once resuspended, store at -20° C, avoid contact with RNases and repeated freeze thaw cycles.

Resuspend lyophilized siRNA duplex in 330 μ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330 μ l of RNase-free water makes a 10 μ M solution in a 10 μ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

Dkk-2 siRNA (h) is recommended for the inhibition of Dkk-2 expression in human cells.

SUPPORT REAGENTS

For optimal siRNA transfection efficiency, Santa Cruz Biotechnology's siRNA Transfection Reagent: sc-29528 (0.3 ml), siRNA Transfection Medium: sc-36868 (20 ml) and siRNA Dilution Buffer: sc-29527 (1.5 ml) are recommended. Control siRNAs or Fluorescein Conjugated Control siRNAs are available as 10 μ M in 66 μ l. Each contain a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA. Fluorescein Conjugated Control siRNAs include: sc-36869, sc-44239, sc-44240 and sc-44241. Control siRNAs include: sc-37007, sc-44230, sc-44231, sc-44232, sc-44233, sc-44234, sc-44235, sc-44236, sc-44237 and sc-44238.

GENE EXPRESSION MONITORING

Dkk-2 (183D2D): sc-517634 is recommended as a control antibody for monitoring of Dkk-2 gene expression knockdown by Western Blotting (starting dilution 1:200, dilution range 1:100-1:1000) or immunofluorescence (starting dilution 1:50, dilution range 1:50-1:500).

To ensure optimal results, the following support reagents are recommended: 1) Western Blotting: use m-IgG κ BP-HRP: sc-516102 or m-IgG κ BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker[™] Molecular Weight Standards: sc-2035, UltraCruz[®] Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-IgG κ BP-FITC: sc-516140 or m-IgG κ BP-PE: sc-516141 (dilution range: 1:50-1:200) with UltraCruz[®] Mounting Medium: sc-24941 or UltraCruz[®] Hard-set Mounting Medium: sc-359850.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor Dkk-2 gene expression knockdown using RT-PCR Primer: Dkk-2 (h)-PR: sc-37084-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.