

frizzled-4 siRNA (m): sc-39984

BACKGROUND

Frizzled-4 is a 537 amino acid protein encoded by the human gene FZD4. Frizzled-4 acts as a receptor for Wnt proteins. Most frizzled receptors are coupled to the β -catenin canonical signaling pathway, which leads to the activation of disheveled proteins, inhibition of GSK-3 kinase, nuclear accumulation of β -catenin and activation of Wnt target genes. A second signaling pathway involving PKC and calcium fluxes has been seen for some family members, but it is not yet clear if it represents a distinct pathway or if it can be integrated in the canonical pathway, as PKC seems to be required for Wnt-mediated inactivation of GSK-3 kinase. Both pathways seem to involve interactions with G proteins. Frizzled-4 may be involved in transduction and intercellular transmission of polarity information during tissue morphogenesis and/or in differentiated tissues. Frizzled-4 also plays a critical role in retinal angiogenesis. Frizzled-4 is virtually ubiquitously expressed with greatest amounts found in adult heart, skeletal muscle, ovary and fetal kidney.

REFERENCES

1. Robitaille, J., et al. 2002. Mutant frizzled-4 disrupts retinal angiogenesis in familial exudative vitreoretinopathy. *Nat. Genet.* 32: 326-330.
2. Omoto, S., et al. 2004. Autosomal dominant familial exudative vitreoretinopathy in two Japanese families with FZD4 mutations (H69Y and C181R). *Ophthalmic Genet.* 25: 81-90.
3. Swain, R.K., et al. 2005. *Xenopus* frizzled-4S, a splicing variant of Xzf4 is a context-dependent activator and inhibitor of Wnt/ β -catenin signaling. *Cell Commun. Signal.* 3: 12.
4. Qin, M., et al. 2005. Complexity of the genotype-phenotype correlation in familial exudative vitreoretinopathy with mutations in the LRP5 and/or FZD4 genes. *Hum. Mutat.* 26: 104-112.
5. MacDonald, M.L., et al. 2005. Genetic variants of frizzled-4 gene in familial exudative vitreoretinopathy and advanced retinopathy of prematurity. *Clin. Genet.* 67: 363-366.
6. Masckauchán, T.N., et al. 2005. Wnt/ β -catenin signaling induces proliferation, survival and interleukin-8 in human endothelial cells. *Angiogenesis* 8: 43-51.

CHROMOSOMAL LOCATION

Genetic locus: Fzd4 (mouse) mapping to 7 E1.

PRODUCT

frizzled-4 siRNA (m) 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 frizzled-4 shRNA Plasmid (m): sc-39984-SH and frizzled-4 shRNA (m) Lentiviral Particles: sc-39984-V as alternate gene silencing products.

For independent verification of frizzled-4 (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-39984A, sc-39984B and sc-39984C.

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

frizzled-4 siRNA (m) is recommended for the inhibition of frizzled-4 expression in mouse 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

frizzled-4 (3G7): sc-293454 is recommended as a control antibody for monitoring of frizzled-4 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 frizzled-4 gene expression knockdown using RT-PCR Primer: frizzled-4 (m)-PR: sc-39984-PR (20 μ l, 446 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

RESEARCH USE

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

PROTOCOLS

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