

# Nectin 4 siRNA (h): sc-62669

## BACKGROUND

Homologous to the poliovirus receptor (PVR/CD155), the nectin immunoglobulin superfamily comprises four known isoforms (-1, -2, -3, and -4). The ecto-domain of nectin family members comprises three Ig-like domains (V, C, C'). Nectins localize at the adherens junctions (AJ) in epithelial and endothelial cells where they serve as adhesion molecules. Actin-based AJs play a role in mechanical adhesion, cellular morphogenesis and cellular differentiation. Nectin associates with the Actin cytoskeleton through its interaction with the Actin filament-binding protein afadin. Nectin 4 and afadin co-localize at cadherin-based adherens junctions in MDCKII epithelial cells. Nectin 4 and Nectin 3 share a common binding region in the V domain of Nectin 1 and thus compete for Nectin 1 binding. The Nectin 3/4 binding domain maps to the C-C'-C"-D  $\beta$  strands of the V domain of Nectin 1. Unlike other nectins, which are more widely expressed, Nectin 4 is mainly expressed in the placenta.

## REFERENCES

1. Reymond, N., et al. 2001. Nectin4/PRR4, a new afadin-associated member of the nectin family that *trans*-interacts with Nectin1/PRR1 through V domain interaction. *J. Biol. Chem.* 276: 43205-43215.
2. Mizoguchi, A., et al. 2002. Nectin: an adhesion molecule involved in formation of synapses. *J. Cell Biol.* 156: 555-565.
3. Fabre, S., et al. 2002. Prominent role of the Ig-like V domain in *trans*-interactions of nectins. Nectin3 and Nectin 4 bind to the predicted C-C'-C"-D  $\beta$ -strands of the Nectin1 V domain. *J. Biol. Chem.* 277: 27006-27013.
4. Ozaki-Kuroda, K., et al. 2002. Nectin couples cell-cell adhesion and the actin scaffold at heterotypic testicular junctions. *Curr. Biol.* 12: 1145-1150.

## CHROMOSOMAL LOCATION

Genetic locus: PVRL4 (human) mapping to 1q23.3.

## PRODUCT

Nectin 4 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  $\mu$ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Nectin 4 shRNA Plasmid (h): sc-62669-SH and Nectin 4 shRNA (h) Lentiviral Particles: sc-62669-V as alternate gene silencing products.

For independent verification of Nectin 4 (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-62669A, sc-62669B and sc-62669C.

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

## APPLICATIONS

Nectin 4 siRNA (h) is recommended for the inhibition of Nectin 4 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  $\mu$ M in 66  $\mu$ 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

Nectin 4 (A-9): sc-515093 is recommended as a control antibody for monitoring of Nectin 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 $\kappa$  BP-HRP: sc-516102 or m-IgG $\kappa$  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 $\kappa$  BP-FITC: sc-516140 or m-IgG $\kappa$  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 Nectin 4 gene expression knockdown using RT-PCR Primer: Nectin 4 (h)-PR: sc-62669-PR (20  $\mu$ l, 556 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

## SELECT PRODUCT CITATIONS

1. Siddharth, S., et al. 2017. Nectin-4 is a breast cancer stem cell marker that induces WNT/ $\beta$ -catenin signaling via Pi3k/Akt axis. *Int. J. Biochem. Cell Biol.* 89: 85-94.

## RESEARCH USE

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

## PROTOCOLS

See our web site at [www.scbt.com](http://www.scbt.com) for detailed protocols and support products.