SANTA CRUZ BIOTECHNOLOGY, INC.

DAN siRNA (h): sc-72320

BACKGROUND

The DAN (differential screening-selected gene aberrative in neuroblastoma) protein family contains antagonists of bone morphogenetic protein (BMP) signaling that are expressed in the neural crest. All family members are secreted proteins that act as BMP antagonists in embryonic explants and are expressed in the proximal airway epithelium of the lung during embryonic development. This family includes the head-inducing factor Cerberus, the dorsaling factor gremlin and the tumor suppressor DAN. DAN, also known as zinc finger protein DAN, NO3, DAND1 or neuroblastoma suppressor of tumorigenicity 1 (NBL1), is a secreted protein containing one C-terminal cysteine knot-like (CTCK) domain. It is produced in small neurons of the dorsal root ganglion and its expression is activated by MATH-1. In addition to antagonizing BMP signaling, DAN also antagonizes the action of TGFβ. DAN is a possible tumor suppressor for human neuroblastoma; defects may result in or contribute to its progression. DAN may also be a neuromodulator in inflammatory pain.

REFERENCES

1. Ohtori, S., et al. 2002. Differential screening-selected gene aberrative in neuroblastoma protein modulates inflammatory pain in the spinal dorsal horn. Neuroscience 110: 579-586.
2. Shinbo, J., et al. 2002. p73-dependent expression of DAN during cisplatin-induced cell death and osteoblast differentiation. Biochem. Biophys. Res. Commun. 295: 501-507.
3. Kim, A.S. and Pleasure, S.J. 2003. Expression of the BMP antagonist Dan during murine forebrain development. Brain Res. Dev. Brain Res. 145: 159-162.
4. Ohtori, S., et al. 2004. Reduced inflammatory pain in mice deficient in the differential screening-selected gene aberrative in neuroblastoma. Mol. Cell. Neurosci. 25: 504-514.
5. Gerlach-Bank, L.M., et al. 2004. DAN directs endolymphatic sac and duct outgrowth in the avian inner ear. Dev. Dyn. 229: 219-230.
6. Hruska, K.A., et al. 2005. Bone morphogenetic proteins in vascular calcification. Circ. Res. 97: 105-114.

CHROMOSOMAL LOCATION

Genetic locus: NBL1 (human) mapping to 1p36.13.

PRODUCT

DAN 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see DAN shRNA Plasmid (h): sc-72320-SH and DAN shRNA (h) Lentiviral Particles: sc-72320-V as alternate gene silencing products.

For independent verification of DAN (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-72320A, sc-72320B and sc-72320C.

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 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

DAN siRNA (h) is recommended for the inhibition of DAN 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 µM in 66 µl. 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

DAN (AT38G8): sc-517397 is recommended as a control antibody for monitoring of DAN 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-IgGκ BP-HRP: sc-516102 or m-IgGκ 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-IgGκ BP-FITC: sc-516140 or m-IgGκ 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 DAN gene expression knockdown using RT-PCR Primer: DAN (h)-PR: sc-72320-PR (20 µl). 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.