

Dia 2 siRNA (h): sc-35192

BACKGROUND

Dia 2, also known as DIAPH2 (diaphanous homolog 2), DRF2 or mDia3 of mouse origin, is a mammalian homolog of the *Drosophila* diaphanous gene, and belongs to a family of formin homology (FH) proteins which are characterized by having tandemly aligned FH1 (formin homology 1) and FH2 (formin homology 2) domains in their carboxy terminal regions. Dia 2 contains a DAD (diaphanous autoregulatory) domain, which is involved in the elongation of Actin filaments, and a GBD/FH3 (Rho GTPase-binding/formin homology 3) domain, which interacts with the DAD domain via autoinhibitory interactions to regulate the activation of Dia 2. Expressed in testis and ovary, Dia 2 may be involved in oogenesis. Defects to the gene encoding Dia 2 have been implicated in premature ovarian failure type 2A, a disorder characterized by decreased ovarian function. Dia 2 exists as three alternatively spliced isoforms.

REFERENCES

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- Bione, S., et al. 1998. A human homologue of the *Drosophila melanogaster* diaphanous gene is disrupted in a patient with premature ovarian failure: evidence for conserved function in oogenesis and implications for human sterility. *Am. J. Hum. Genet.* 62: 533-541.
- Alberts, A.S., et al. 1998. Analysis of RhoA-binding proteins reveals an interaction domain conserved in heterotrimeric G protein β subunits and the yeast response regulator protein Skn7. *J. Biol. Chem.* 273: 8616-8622.
- Watanabe, N., et al. 1999. Cooperation between mDia1 and ROCK in Rho-induced Actin reorganization. *Nat. Cell Biol.* 1: 136-143.
- Nakano, K., et al. 1999. Distinct actions and cooperative roles of ROCK and mDia in Rho small G protein-induced reorganization of the Actin cytoskeleton in Madin-Darby canine kidney cells. *Mol. Biol. Cell* 10: 2481-2491.
- Afshar, K., et al. 2000. Functional analysis of the *Drosophila* diaphanous FH protein in early embryonic development. *Development* 127: 1887-1897.

CHROMOSOMAL LOCATION

Genetic locus: DIAPH2 (human) mapping to Xq21.33.

PRODUCT

Dia 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 Dia 2 shRNA Plasmid (h): sc-35192-SH and Dia 2 shRNA (h) Lentiviral Particles: sc-35192-V as alternate gene silencing products.

For independent verification of Dia 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-35192A, sc-35192B and sc-35192C.

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

Dia 2 siRNA (h) is recommended for the inhibition of Dia 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

Dia 2 (H-3): sc-393489 is recommended as a control antibody for monitoring of Dia 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 Dia 2 gene expression knockdown using RT-PCR Primer: Dia 2 (h)-PR: sc-35192-PR (20 μ l, 410 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.