

# DIAPH3 siRNA (h): sc-62210

## BACKGROUND

DIAPH3 (diaphanous homolog 3), also known as DIAP3 or DRF3, is a 1,193 amino acid member of the formin homology protein family and is required for the correct function of various cellular processes. DIAPH3 binds to both Profilin, a protein involved in cell maintenance, and to the GTP-bound form of Rho (Rho GTP). Binding to both of these proteins allows DIAPH3 to recruit Profilin to the membrane in a Rho-dependent manner. At the membrane, DIAPH3 promotes actin polymerization and is required for stress fiber formation, cytokinesis and transcriptional activation of the serum response factor (SRF). DIAPH3 also regulates actin dynamics by coupling Src tyrosine kinase (c-Src) and Rho during Actin signaling events. DIAPH3 contains one diaphanous autoregulatory domain (DAD) and one Rho GTPase-binding domain (GBD). When DAD and GBD are intramolecularly bound, the GBD is occupied and DIAPH3 is inactive. Interruption of the DAD-GBD bond allows the GBD to bind to Rho GTP, thus activating DIAPH3. Seven isoforms of DIAPH3 exist due to alternative splicing events.

## REFERENCES

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2. Katoh, M. and Katoh, M. 2004. Identification and characterization of human DIAPH3 gene in silico. *Int. J. Mol. Med.* 13: 473-478.
3. Katoh, M. and Katoh, M. 2004. Identification and characterization of human FHOD3 gene in silico. *Int. J. Mol. Med.* 13: 615-620.
4. Katoh, M. and Katoh, M. 2004. Identification and characterization of human FHDC1, mouse Fhdc1 and zebrafish fhdc1 genes in silico. *Int. J. Mol. Med.* 13: 929-934.
5. Katoh, Y. and Katoh, M. 2004. Identification and characterization of CDC50A, CDC50B and CDC50C genes in silico. *Oncol. Rep.* 12: 939-943.

## CHROMOSOMAL LOCATION

Genetic locus: DIAPH3 (human) mapping to 13q21.2.

## PRODUCT

DIAPH3 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 DIAPH3 shRNA Plasmid (h): sc-62210-SH and DIAPH3 shRNA (h) Lentiviral Particles: sc-62210-V as alternate gene silencing products.

For independent verification of DIAPH3 (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-62210A, sc-62210B and sc-62210C.

## PROTOCOLS

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

## 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

DIAPH3 siRNA (h) is recommended for the inhibition of DIAPH3 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

DIAPH3 (4D5): sc-293288 is recommended as a control antibody for monitoring of DIAPH3 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 DIAPH3 gene expression knockdown using RT-PCR Primer: DIAPH3 (h)-PR: sc-62210-PR (20  $\mu$ l). 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.