

# rhophilin siRNA (h): sc-39221

## BACKGROUND

Rho, the Ras-related small GTPase, is responsible for the regulation of Actin-based cytoskeletal structures including stress fibers, focal adhesions and the contractile ring apparatus. Rho proteins act as molecular switches which are able to turn cytokinesis on and off. Although little is known about signaling downstream of Rho, several proteins have been implicated as Rho effectors. Protein kinase N (PKN) is a fatty acid-activated serine/threonine kinase whose catalytic domain exhibits homology with that of the PKC family. PKN associates with Rho via its amino-terminus, is activated in a GTP-dependent manner and phosphorylates the head-rod domain of neurofilament protein. A second protein, rhophilin, exhibits 40% sequence identity with the amino-terminal Rho binding domain. The enzymatic activity of rhophilin has not been demonstrated and it is possible that it acts through the recruitment of cytoskeletal components that initiate a kinase signaling cascade. Citron interacts specifically with active Rho and Rac 1 but not Cdc42. Citron exhibits a distinctive protein organization and little homology with the Rho binding domains of PKN and rhophilin.

## REFERENCES

1. Kitagawa, M., et al. 1995. Purification and characterization of a fatty acid-activated protein kinase (PKN) from rat testis. *Biochem. J.* 310: 657-664.
2. Madaule, P., et al. 1995. A novel partner for the GTP-bound forms of Rho and Rac. *FEBS Lett.* 377: 243-248.
3. Shibata, H., et al. 1996. Characterization of the interaction between RhoA and the amino-terminal region of PKN. *FEBS Lett.* 385: 221-224.
4. Watanabe, G., et al. 1996. Protein kinase N (PKN) and PKN-related protein rhophilin as targets of small GTPase Rho. *Science* 271: 645-648.
5. Amano, M., et al. 1996. Identification of a putative target for Rho as the serine-threonine kinase protein kinase N. *Science* 271: 648-650.
6. Mukai, H., et al. 1996. PKN associates and phosphorylates the head-rod domain of neurofilament protein. *J. Biol. Chem.* 271: 9816-9822.
7. Kitagawa, M., et al. 1996. The role of the unique motifs in the amino-terminal region of PKN on its enzymatic activity. *Biochem. Biophys. Res. Commun.* 220: 963-968.

## CHROMOSOMAL LOCATION

Genetic locus: RHPN1 (human) mapping to 8q24.3.

## PRODUCT

rhophilin 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 rhophilin shRNA Plasmid (h): sc-39221-SH and rhophilin shRNA (h) Lentiviral Particles: sc-39221-V as alternate gene silencing products.

For independent verification of rhophilin (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-39221A, sc-39221B and sc-39221C.

## 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

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

rhophilin (F-7): sc-166302 is recommended as a control antibody for monitoring of rhophilin 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<sup>™</sup> Molecular Weight Standards: sc-2035, UltraCruz<sup>®</sup> 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<sup>®</sup> Mounting Medium: sc-24941 or UltraCruz<sup>®</sup> Hard-set Mounting Medium: sc-359850.

## RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor rhophilin gene expression knockdown using RT-PCR Primer: rhophilin (h)-PR: sc-39221-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.

## PROTOCOLS

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