SANTA CRUZ BIOTECHNOLOGY, INC.

rhophilin-2 siRNA (m): sc-62939

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 proteins. 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

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2. Kitagawa, M., Mukai, H., Shibata, H. and Ono, Y. 1995. Purification and characterization of a fatty acid-activated protein kinase (PKN) from rat testis. Biochem. J. 310: 657-664.
3. Mukai, H., Toshimori, M., Shibata, H., Kitagawa, M., Shimakawa, M., Miyahara, M., Sunakawa, H. and Ono, Y. 1996. PKN associates and phosphorylates the head-rod domain of neurofilament protein. J. Biol. Chem. 271: 9816-9822.
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5. Kitagawa, M., Shibata, H., Toshimori, M., Mukai, H. and Ono, Y. 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.
6. Watanabe, G., Saito, Y., Madaule, P., Ishizaki, T., Fujisawa, K., Morii, N., Mukai, H., Ono, Y., Kakizuka, A. and Narumiya, S. 1996. Protein kinase N (PKN) and PKN-related protein rhophilin as targets of small GTPase Rho. Science 271: 645-648.
7. Amano, M., Mukai, H., Ono, Y., Chihara, K., Matsui, T., Hamajima, Y., Okawa, K., Iwamatsu, A. and Kaibuchi, K. 1996. Identification of a putative target for Rho as the serine-threonine kinase protein kinase N. Science 271: 648-650.

CHROMOSOMAL LOCATION

Genetic locus: Rhpn2 (mouse) mapping to 7 B1.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.

PRODUCT

rhophilin-2 siRNA (m) 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 rhophilin-2 shRNA Plasmid (m): sc-62939-SH and rhophilin-2 shRNA (m) Lentiviral Particles: sc-62939-V as alternate gene silencing products.

For independent verification of rhophilin-2 (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-62939A, sc-62939B and sc-62939C.

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

rhophilin-2 siRNA (m) is recommended for the inhibition of rhophilin-2 expression in mouse 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.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor rhophilin-2 gene expression knockdown using RT-PCR Primer: rhophilin-2 (m)-PR: sc-62939-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Yang, J., Wu, C., Stefanescu, I., Jakobsson, L., Chervoneva, I. and Horowitz, A. 2016. RhoA inhibits neural differentiation in murine stem cells through multiple mechanisms. Sci. Signal. 9: ra76.

RESEARCH USE

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