SANTA CRUZ BIOTECHNOLOGY, INC.

Rock-1 siRNA (r): sc-72179

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 function as molecular switches that are able to turn cytokinesis on and off. Although little is known about signaling downstream of Rho, a host of putative Rho effector proteins have been described, including rhophilin, Rhotekin, citron and the serine/threonine kinase, protein kinase N. Two additional Rho-activated serine/threonine kinases have been described, designated Rock-1 and Rock-2 (also referred to as Roka, for Rho-associated coil-containing protein kinase). Rock-1 and Rock-2 share a structural similarity with myotonic dystrophy kinase.

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. Leung, T., et al. 1995. A novel serine/threonine kinase binding the Ras-related Rho A GTPase which translocates the kinase to peripheral membranes. J. Biol. Chem. 270: 29051-29054.
3. Amano, M., et al. 1996. Identification of a putative target for Rho as the serine-threonine kinase protein kinase N. Science 271: 648-650.
4. Mukai, H., et al. 1996. PKN associates and phosphorylates the head-rod domain of neurofilament protein. J. Biol. Chem. 271: 9816-9822.
5. Shibata, H., et al. 1996. Characterization of the interaction between Rho A and the amino-terminal region of PKN. FEBS Lett. 385: 221-224.
6. 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.
7. Watanabe, G., et al. 1996. Protein kinase N (PKN) and PKN-related protein rhophilin as targets of small GTPase Rho. Science 271: 645-648.
8. Ishizaki, T., et al. 1996. The small GTP-binding protein Rho binds to and activates a 160 kDa Ser/Thr protein kinase homologous to myotonic dystrophy kinase. EMBO J. 15: 1885-1893.

CHROMOSOMAL LOCATION

Genetic locus: Rock1 (rat) mapping to 18p13.

PRODUCT

Rock-1 siRNA (r) 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 Rock-1 shRNA Plasmid (r): sc-72179-SH and Rock-1 shRNA (r) Lentiviral Particles: sc-72179-V as alternate gene silencing products.

For independent verification of Rock-1 (r) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-72179A, sc-72179B and sc-72179C.

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

Rock-1 siRNA (r) is recommended for the inhibition of Rock-1 expression in rat 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

Rock-1 (G-6): sc-17794 is recommended as a control antibody for monitoring of Rock-1 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 Rock-1 gene expression knockdown using RT-PCR Primer: Rock-1 (r)-PR: sc-72179-PR (20 µl, 567 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Ávila-Rodríguez, D., et al. 2017. The shift in GH3 cell shape and cell motility is dependent on MLCK and ROCK. Exp. Cell Res. 354: 1-17.

RESEARCH USE

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