

Rock-1 siRNA (h): sc-29473

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 rhotillin, 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.

CHROMOSOMAL LOCATION

Genetic locus: ROCK1 (human) mapping to 18q11.1.

PRODUCT

Rock-1 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 Rock-1 shRNA Plasmid (h): sc-29473-SH and Rock-1 shRNA (h) Lentiviral Particles: sc-29473-V as alternate gene silencing products.

For independent verification of Rock-1 (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-29473A, sc-29473B and sc-29473C.

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

Rock-1 siRNA (h) is recommended for the inhibition of Rock-1 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.

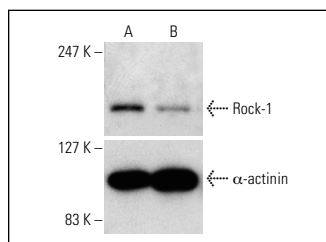
GENE EXPRESSION MONITORING

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).

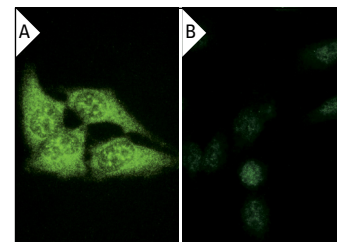
RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor Rock-1 gene expression knockdown using RT-PCR Primer: Rock-1 (h)-PR: sc-29473-PR (20 μ l, 472 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

DATA



Rock-1 siRNA (h): sc-29473. Western blot analysis of Rock-1 expression in non-transfected control (A) and Rock-1 siRNA transfected (B) HeLa cells. Blot probed with Rock-1 (G-6): sc-17794. α -actinin (H-2): sc-17829 used as specificity and loading control.



Rock-1 siRNA (h): sc-29473. Immunofluorescence staining of methanol-fixed, control HeLa (A) and Rock-1 siRNA silenced HeLa (B) cells showing diminished cytoskeletal staining in the siRNA silenced cells. Cells probed with Rock-1 (G-6): sc-17794.

SELECT PRODUCT CITATIONS

- Shao, J., et al. 2008. ROCK and PRK-2 mediate the inhibitory effect of Y-27632 on polyglutamine aggregation. *FEBS Lett.* 582: 1637-1642.
- Neill, T., et al. 2013. Decorin induces rapid secretion of thrombospondin-1 in basal breast carcinoma cells via inhibition of Ras homolog gene family, member A/Rho-associated coiled-coil containing protein kinase 1. *FEBS J.* 280: 2353-2368.
- Deng, J.T., et al. 2015. The effects of knockdown of Rho-associated kinase 1 and zipper-interacting protein kinase on gene expression and function in cultured human arterial smooth muscle cells. *PLoS ONE* 10: e0116969.
- Heemskerk, N., et al. 2016. F-Actin-rich contractile endothelial pores prevent vascular leakage during leukocyte diapedesis through local RhoA signalling. *Nat. Commun.* 7: 10493.
- Wilson, J.L., et al. 2018. Unraveling endothelin-1 induced hypercontractility of human pulmonary artery smooth muscle cells from patients with pulmonary arterial hypertension. *PLoS ONE* 13: e0195780.
- Takeda, Y., et al. 2019. Rock2 regulates monocyte migration and cell to cell adhesion in vascular endothelial cells. *Int. J. Mol. Sci.* 20 pii: E1331.

RESEARCH USE

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