SANTA CRUZ BIOTECHNOLOGY, INC.

arfaptin 1 siRNA (h): sc-41190

BACKGROUND

ADP-ribosylation factors, or ARFs, enhance the ADP ribosyltransferase activity of cholera toxin and are implicated in vesicle transport between endoplasmic reticulum and the Golgi complex. Arfaptin 1 is recruited from the cytosol to Golgi membranes by ARFs in a guanosine 5'-O-(3-thiotriphosphate)-dependent and brefeldin A-sensitive manner but is not a constituent of coatomer. Arfaptin 1 binds to nonmyristoylated GTP-bound ARF3, but not to GDP-bound ARF3, and also to ARF1, another class I ARF. It binds with lower affinity to ARF5, a class II ARF, and with very little affinity to ARF6, a class III ARF. POR1 (also designated Arfaptin 2) was first isolated as a Rac 1 binding protein necessary for Rac mediated actin polymerization and the subsequent formation of membrane ruffles and lamellipodia. POR1 has also been shown to interact with the ADP ribosylation factor ARF6, a GTPase that associates with the plasma membrane and intracellular endosome vesicles, in a GTP dependent manner. The association of POR1 with ARF6 stimulates induction of actin polymerization. POR1 appears to play a regulatory role through multiple signaling pathways in the reorganization of the cytoskeletal structure.

REFERENCES

1. Joneson, T., et al. 1996. Rac regulation of actin polymerization and proliferation by a pathway distinct from Jun kinase. Science 274: 1374-1376.
2. Van Aelst, L., et al. 1996. Identification of a novel Rac 1-interacting protein involved in membrane ruffling. EMBO J. 15: 3778-3786.
3. Kanoh, H., et al. 1997. Arfaptin 1, a putative cytosolic target protein of ADP-ribosylation factor, is recruited to Golgi membranes. J. Biol. Chem. 272: 5421-5429.
4. D'Souza-Schorey, C., et al. 1997. A role for POR1, a Rac 1-interacting protein, in ARF6-mediated cytoskeletal rearrangements. EMBO J. 16: 5445-5454.
5. D'Souza-Schorey, C., et al. 1998. ARF6 targets recycling vesicles to the plasma membrane: insights from an ultrastructural investigation. J. Cell Biol. 140: 603-616.
6. Gauthier-Rouviere, C., et al. 1998. RhoG GTPase controls a pathway that independently activates Rac1 and Cdc42Hs. Mol. Biol. Cell 9: 1379-1394.

CHROMOSOMAL LOCATION

Genetic locus: ARFIP1 (human) mapping to 4q31.3.

PRODUCT

arfaptin 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see arfaptin 1 shRNA Plasmid (h): sc-41190-SH and arfaptin 1 shRNA (h) Lentiviral Particles: sc-41190-V as alternate gene silencing products.

For independent verification of arfaptin 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-41190A, sc-41190B and sc-41190C.

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

arfaptin 1 siRNA (h) is recommended for the inhibition of arfaptin 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 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

arfaptin 1 (F-3): sc-374361 is recommended as a control antibody for monitoring of arfaptin 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 arfaptin 1 gene expression knockdown using RT-PCR Primer: arfaptin 1 (h)-PR: sc-41190-PR (20 µl). 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 for detailed protocols and support products.