

POSH siRNA (m): sc-36294

BACKGROUND

Rho, Rac and Cdc42 are members of the small GTPase family. These proteins act as molecular switches, cycling between an active GTP-bound state and an inactive GDP-bound state. Activation of these proteins results in rearrangements of filamentous actin and the formation of actin stress fibers. Many of the targets of these GTPases are involved in signal transduction events mediated by Src3 homology (SH3) domains. POSH, for "plenty of SH3s", is a Rac binding protein with four SH3 domains. POSH preferentially interacts with the GTP form of Rac and not with the GDP-bound Rac. Ectopic expression of POSH elicits JNK activation and nuclear translocation of NFκB, suggesting that POSH is involved in Rac regulation of these kinase pathways. Overexpression of POSH has also been shown to induce apoptosis.

REFERENCES

1. Ridley, A.J. and Hall, A. 1992. The small GTP-binding protein Rho regulates the assembly of focal adhesions and Actin stress fibers in response to growth factors. *Cell* 70: 389-399.
2. Van Aelst, L. and D'Souza-Schorey, C. 1997. Rho GTPases and signaling networks. *Genes Dev.* 11: 2295-2322.
3. Knaus, U.G. and Bokoch, G.M. 1998. The p21Rac/Cdc42-activated kinases (PAKs). *Int. J. Biochem. Cell Biol.* 30: 857-862.
4. Sudol, M. 1998. From Src Homology domains to other signaling modules: proposal of the "protein recognition code". *Oncogene* 17: 1469-1474.
5. Tapon, N., Nagata, K., Lamarche, N. and Hall, A. 1998. A new Rac target POSH is an SH3-containing scaffold protein involved in the JNK and NFκB signalling pathways. *EMBO J.* 17: 1395-1404.
6. Sasaki, T. and Takai, Y. 1998. The Rho small G protein family-Rho GDI system as a temporal and spatial determinant for cytoskeletal control. *Biochem. Biophys. Res. Commun.* 245: 641-645.

CHROMOSOMAL LOCATION

Genetic locus: Sh3rf1 (mouse) mapping to 8 B3.1.

PRODUCT

POSH 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 POSH shRNA Plasmid (m): sc-36294-SH and POSH shRNA (m) Lentiviral Particles: sc-36294-V as alternate gene silencing products.

For independent verification of POSH (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-36294A, sc-36294B and sc-36294C.

PROTOCOLS

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

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

POSH siRNA (m) is recommended for the inhibition of POSH 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.

GENE EXPRESSION MONITORING

POSH (A-4): sc-376059 is recommended as a control antibody for monitoring of POSH 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 POSH gene expression knockdown using RT-PCR Primer: POSH (m)-PR: sc-36294-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.