SANTA CRUZ BIOTECHNOLOGY, INC.

PKN siRNA (h): sc-36261

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 know 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 protein. 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 Rac1 but not Cdc42. Citron exhibits a distinctive protein organization and little homology with the Rho binding domains of PKN and rhophilin.

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. Madaule, P., et al. 1995. A novel partner for the GTP-bound forms of Rho and Rac. FEBS Lett. 377: 243-248.
3. Watanabe, G., et al. 1996. Protein kinase N (PKN) and PKN-related protein rhophilin as targets of small GTPase Rho. Science 271: 645-648.
4. Amano, M., et al. 1996. Identification of a putative target for Rho as the serine-threonine kinase protein kinase N. Science 271: 648-650.
5. Mukai, H., et al. 1996. PKN associates and phosphorylates the head-rod domain of neurofilament protein. J. Biol. Chem. 271: 9816-9822.
6. Shibata, H., et al. 1996. Characterization of the interaction between RhoA and the amino-terminal region of PKN. FEBS Lett. 385: 221-224.
7. 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.

CHROMOSOMAL LOCATION

Genetic locus: PKN1 (human) mapping to 19p13.12.

PRODUCT

PKN siRNA (h) is a target-specific 19-25 nt siRNA 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 PKN shRNA Plasmid (h): sc-36261-SH and PKN shRNA (h) Lentiviral Particles: sc-36261-V as alternate gene silencing products.

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

PKN siRNA (h) is recommended for the inhibition of PKN 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

PKN (H-4): sc-393344 is recommended as a control antibody for monitoring of PKN 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 PKN gene expression knockdown using RT-PCR Primer: PKN (h)-PR: sc-36261-PR (20 µl, 424 bp). 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.