SANTA CRUZ BIOTECHNOLOGY, INC.

ROPN1 siRNA (h): sc-78553

BACKGROUND

The type II cAMP-dependent protein kinase (PKA) is a multifunctional kinase with a broad range of substrates. Specificity of PKA signaling is mediated by the compartmentalization of the kinase to specific sites within the cell. To maintain this specific localization, the R subunit (RII) of PKA interacts with specific RII-anchoring proteins, designated A-kinase anchoring proteins (AKAP). AKAP 3, also known as AKAP 110, FSP95, PRKA3 and SOB1, binds both PKA and PDE4A and functions as a scaffolding protein in spermatozoa to regulate local cAMP concentrations and modulate sperm functions. Expression of AKAP 3 in normal tissues is restricted to the testis, where bicarbonate stimulates tyrosine phosphorylation of AKAP 3, thereby increasing its recruitment of PKA. AKAP 3 serves as an anchoring protein for ROPN1, also designated Ropporin. ROPN1 expression is limited to testis and fetal liver in normal tissues, but can also be detected in multiple myeloma, chronic lymphocytic leukemia and acute myeloid leukemia tumor cells. ROPN1 forms a complex with rhophilin in sperm flagella to mediate its function.

REFERENCES

1. Scott, J.D., et al. 1990. Type II regulatory subunit dimerization determines the subcellular localization of the cAMP-dependent protein kinase. J. Biol. Chem. 265: 21561-21566.
2. Coghlan, V.M., et al. 1993. A-kinase anchoring proteins: a key to selective activation of cAMP-responsive events? Mol. Cell. Biochem. 127: 309-319.
3. Coghlan, V.M., et al. 1995. Association of protein kinase A and protein phosphatase 2B with a common anchoring protein. Science 267: 108-111.
4. Fujita, A., et al. 2000. Ropporin, a sperm-specific binding protein of rhophilin, that is localized in the fibrous sheath of sperm flagella. J. Cell Sci. 113: 103-112.
5. Eddy, E.M., et al. 2003. Fibrous sheath of mammalian spermatozoa. Microsc. Res. Tech. 61: 103-115.
6. Li, Z., et al. 2007. A yeast two-hybrid system using Sp17 identified Ropporin as a novel cancer-testis antigen in hematologic malignancies. Int. J. Cancer 121: 1507-1511.

CHROMOSOMAL LOCATION

Genetic locus: ROPN1 (human) mapping to 3q21.1.

PRODUCT

ROPN1 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 ROPN1 shRNA Plasmid (h): sc-78553-SH and ROPN1 shRNA (h) Lentiviral Particles: sc-78553-V as alternate gene silencing products.

For independent verification of ROPN1 (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-78553A, sc-78553B and sc-78553C.

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

ROPN1 siRNA (h) is recommended for the inhibition of ROPN1 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

ROPN1 (IW.63): sc-130455 is recommended as a control antibody for monitoring of ROPN1 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 ROPN1 gene expression knockdown using RT-PCR Primer: ROPN1 (h)-PR: sc-78553-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.