

RABAPTIN-5 siRNA (h): sc-36349

BACKGROUND

The Ras-related superfamily of guanine nucleotide binding proteins, which includes the R-Ras, Rap, Ral/Rec and Rho/Rab subfamilies, exhibits 30-60% homology with Ras p21. Accumulating data suggests an important role for Rab proteins, either in endocytosis or in biosynthetic protein transport. The transport of newly synthesized proteins from the endoplasmic reticulum to various stacks of the Golgi complex and to secretory vesicles involves at each stage the movement of carrier vesicles, a process that appears to involve Rab protein function. Rab proteins are also an integral part of endocytic pathways. For example, Rab 5 is a potent regulator of endocytic transport that is localized to the plasma membrane, clathrin coated pits and early endosomes. A possible downstream effector of Rab 5, designated RABAPTIN-5, is a coiled-coil protein that physically associates with Rab 5. Immunodepletion of RABAPTIN-5 strongly inhibits Rab5-dependent early endosome fusion and may be required for membrane docking and fusion.

REFERENCES

1. Zahraoui, A., et al. 1989. The human Rab genes encode a family of GTP-binding proteins related to yeast YPT1 and Sec4 products involved in secretion. *J. Biol. Chem.* 264: 12394-12401.
2. Baldini, G., et al. 1992. Cloning of a Rab 3 isotype predominately expressed in adipocytes. *Proc. Natl. Acad. Sci. USA* 89: 5049-5052.
3. Pfeffer, S.R. 1992. GTP-binding proteins in intracellular transport. *Trends Cell Biol.* 2: 41-46.
4. Takizawa, P., et al. 1993. Coatomers and SNAREs in promoting membrane traffic. *Cell* 75: 593-596.

CHROMOSOMAL LOCATION

Genetic locus: RABEP1 (human) mapping to 17p13.2.

PRODUCT

RABAPTIN-5 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 RABAPTIN-5 shRNA Plasmid (h): sc-36349-SH and RABAPTIN-5 shRNA (h) Lentiviral Particles: sc-36349-V as alternate gene silencing products.

For independent verification of RABAPTIN-5 (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-36349A, sc-36349B and sc-36349C.

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

RABAPTIN-5 siRNA (h) is recommended for the inhibition of RABAPTIN-5 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

RABAPTIN-5 (B-8): sc-271069 is recommended as a control antibody for monitoring of RABAPTIN-5 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-IgG κ BP-HRP: sc-516102 or m-IgG κ 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-IgG κ BP-FITC: sc-516140 or m-IgG κ 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 RABAPTIN-5 gene expression knockdown using RT-PCR Primer: RABAPTIN-5 (h)-PR: sc-36349-PR (20 μ l, 565 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Vela, E.M., et al. 2008. Pichindé virus is trafficked through a dynamin 2 endocytic pathway that is dependent on cellular Rab5- and Rab7-mediated endosomes. *Arch. Virol.* 153: 1391-1396.
2. Wang, Y., et al. 2009. Regulation of endocytosis via the oxygen-sensing pathway. *Nat. Med.* 15: 319-324.

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.