

Rab 5A siRNA (r): sc-156057

BACKGROUND

The Ras-related superfamily of guanine nucleotide binding proteins, which includes the R-Ras, RAP, Ral/Rec and Rho/Rab subfamilies, exhibit 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. The possibility that Rab proteins might also direct the exocytosis from secretory vesicles to the plasma membrane is supported by the observation that in yeast, the Sec4 protein, which is 40% homologous to Rab proteins, is associated with secretory vesicles. At least eight members of the Rab subfamily have been identified, each of which is found at a particular stage of a membrane transport pathway.

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. Chavrier, P., et al. 1992. The complexity of the Rab and Rho GTP-binding protein subfamilies revealed by a PCR cloning approach. *Gene* 112: 261-264.
4. Chen, Y., et al. 1993. Expression and localization of two low molecular weight GTP-binding proteins, Rab 8 and Rab 10, by epitope tag. *Proc. Natl. Acad. Sci. USA* 90: 6508-6512.
5. Torti, M., et al. 1993. Association of the low molecular weight GTP-binding protein Rap 2B with the cytoskeleton during platelet aggregation. *Proc. Natl. Acad. Sci. USA* 90: 7553-7557.
6. Karniguian, A., et al. 1993. Identification of small GTP-binding Rab proteins in human platelets: Thrombin-induced phosphorylation of Rab 3B, Rab 6, and Rab 8 proteins. *Proc. Natl. Acad. Sci. USA* 90: 7647-7651.
7. Takizawa, P. and Malhotra, V. 1993. Coatomers and SNAREs in promoting membrane traffic. *Cell* 75: 593-596.

CHROMOSOMAL LOCATION

Genetic locus: Rab5a (rat) mapping to 9q11.

PRODUCT

Rab 5A siRNA (r) is a pool of 2 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 Rab 5A shRNA Plasmid (r): sc-156057-SH and Rab 5A shRNA (r) Lentiviral Particles: sc-156057-V as alternate gene silencing products.

For independent verification of Rab 5A (r) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-156057A and sc-156057B.

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

Rab 5A siRNA (r) is recommended for the inhibition of Rab 5A expression in rat 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

Rab 5 (D-11): sc-46692 is recommended as a control antibody for monitoring of Rab 5A 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 Rab 5A gene expression knockdown using RT-PCR Primer: Rab 5A (r)-PR: sc-156057-PR (20 μ l). 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.