



Rab 7 siRNA (h):sc-29460



The Power to Question

BACKGROUND

The Ras-related superfamily of guanine nucleotide binding proteins, which includes the Ral/Rec, Rap, R-Ras 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. Several members of the Rab subfamily have been identified, each of which is found at a particular stage of a membrane transport pathway.

REFERENCES

1. 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.
2. Baldini, G., et al. 1992. Cloning of a Rab3 isotype predominately expressed in adipocytes. *Proc. Natl. Acad. Sci. USA* 89: 5049-5052.
3. Novick, P. and Brennwald, P. 1993. Friends and family: the role of the Rab GTPases in vesicular traffic. *Cell* 75: 597-601.
4. Feng, Y., et al. 1995. Rab 7: an important regulator of late endocytic membrane traffic. *J. Cell. Biol.* 131: 1435-1452.
5. Soldati, T., et al. 1995. Rab7 and Rab9 are recruited onto late endosomes by biochemically distinguishable processes. *J. Biol. Chem.* 270: 25541-25548.

CHROMOSOMAL LOCATION

Genetic locus: RAB7 (human) mapping to 3q21; Rab7 (mouse) mapping to 6 D1.

PRODUCT

Rab 7 siRNA (h) is a pool of 3 target-specific 20-25 nt siRNAs designed to knock down gene expression. Each vial contains 3 nmol of lyophilized siRNA, sufficient for a 10 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections.

For independent verification of Rab 7 (h) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3 nmol of lyophilized siRNA. These include: sc-29460A, sc-29460B and sc-29460C.

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

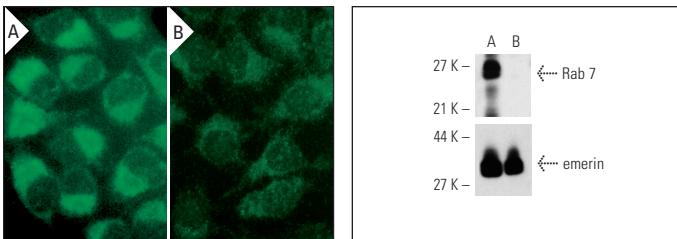
Rab 7 siRNA (h) is recommended for the inhibition of Rab 7 expression in human cells.

Rab 7 (H-50): sc-10767 is recommended as a control antibody for Western Blotting (starting dilution 1:100, dilution range 1:100-1:1,000) or immuno-3fluorescence (starting dilution 1:50, dilution range 1:50-1:500) protein detection using the recommended secondary reagents listed below.

RECOMMENDED SECONDARY REAGENTS

To ensure optimal results, the following support (secondary) reagents are recommended: 1) Western Blotting: use goat anti-rabbit IgG-HRP: sc-2004 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible goat anti-rabbit IgG-HRP: sc-2030 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-rabbit IgG-FITC: sc-2012 (dilution range: 1:100-1:400) or goat anti-rabbit IgG-TR: sc-2780 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

DATA



Rab 7 siRNA (h): sc-29460. Immunofluorescence staining of methanol-fixed, control HeLa (**A**) and Rab 7 siRNA silenced HeLa (**B**) cells showing diminished cytoplasmic staining in the siRNA silenced cells. Cells probed with Rab 7 (H-50): sc-10767.

Rab 7 siRNA (h): sc-29460. Western blot analysis of Rab 7 expression in non-transfected control (**A**) and Rab 7 siRNA transfected (**B**) HeLa cells. Blot probed with Rab 7 (H-50): sc-10767, emerin (H-12): sc-25284 used as specificity and loading control.

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 60 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. Semi-quantitative RT-PCR may be performed using RT-PCR Primer: Rab 7 (h)-PR: sc-29460-PR (20 μ l, 400 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.