

Rab 25 siRNA (h): sc-76325

BACKGROUND

The Ras-related superfamily of guanine nucleotide binding proteins includes the R-Ras, Rap, Ral/Rec and Rho/Rab subfamilies. Increasing data suggests an important role for Rab proteins in either endocytosis or in biosynthetic protein transport. The process of transporting newly synthesized proteins from the endoplasmic reticulum to various stacks of the Golgi complex and to secretory vesicles involves the movement of carrier vesicles and requires Rab protein function. Rab proteins are also an integral part of endocytic pathways. Rab 25, also known as CATX-8 or Rab 11C, is a member of the Rab family of proteins that is exclusively expressed in epithelial cells and participates in apical vesicle trafficking. Rab 25 is overexpressed in ovarian and breast cancer cells and has been associated with metastasis and tumor aggressiveness. The forced expression of Rab 25 in ovarian and breast cancer cells increases cell proliferation *in vivo*.

REFERENCES

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3. 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.
4. 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.
5. Novick, P. and Brennwald, P. 1993. Friends and family: the role of the Rab GTPases in vesicular traffic. *Cell* 75: 597-601.
6. Feng, Y., et al. 1995. Rab 7: an important regulator of late endocytic membrane traffic. *J. Cell Biol.* 131: 1435-1452.
7. Soldati, T., et al. 1995. Rab 7 and Rab 9 are recruited onto late endosomes by biochemically distinguishable processes. *J. Biol. Chem.* 270: 25541-25548.

CHROMOSOMAL LOCATION

Genetic locus: RAB25 (human) mapping to 1q22.

PRODUCT

Rab 25 siRNA (h) 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 25 shRNA Plasmid (h): sc-76325-SH and Rab 25 shRNA (h) Lentiviral Particles: sc-76325-V as alternate gene silencing products.

For independent verification of Rab 25 (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-76325A and sc-76325B.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

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 25 siRNA (h) is recommended for the inhibition of Rab 25 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

Rab 25 (3F12F3): sc-65978 is recommended as a control antibody for monitoring of Rab 25 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 25 gene expression knockdown using RT-PCR Primer: Rab 25 (h)-PR: sc-76325-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.