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# Rab 6 shRNA (h) Lentiviral Particles: sc-44063-V

## 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. Rab 6, also known as RAB6B, RAB6A or RAB6A, is a 208 amino acid protein that is ubiquitously expressed and is a member of the small GTPase superfamily.

## 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 sub-families revealed by a PCR cloning approach. *Gene* 112: 261-264.
4. Takizawa, P. et al. 1993. Coatomers and SNAREs in promoting membrane traffic. *Cell* 75: 593-596.
5. Novick, P., et al. 1993. Friends and family: the role of the Rab GTPases in vesicular traffic. *Cell* 75: 597-601.

## PRODUCT

Rab 6 shRNA (h) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200  $\mu$ l frozen stock containing  $1.0 \times 10^6$  infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see Rab 6 siRNA (h): sc-44063 and Rab 6 shRNA Plasmid (h): sc-44063-SH as alternate gene silencing products.

## STORAGE

Store lentiviral particles at  $-80^{\circ}$  C. Stable for at least one year from the date of shipment. Once thawed, particles can be stored at  $4^{\circ}$  C for up to one week. Avoid repeated freeze thaw cycles.

## APPLICATIONS

Rab 6 shRNA (h) Lentiviral Particles is recommended for the inhibition of Rab 6A, Rab 6B and Rab 6C expression in human cells.

## SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200  $\mu$ l frozen viral stock containing  $1.0 \times 10^6$  infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

## GENE EXPRESSION MONITORING

Rab 6 (K-15): sc-26574 is recommended as a control antibody for monitoring of Rab 6 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 (secondary) reagents are recommended: 1) Western Blotting: use donkey anti-goat IgG-HRP: sc-2020 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible donkey anti-goat IgG-HRP: sc-2033 (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 donkey anti-goat IgG-FITC: sc-2024 (dilution range: 1:100-1:400) or donkey anti-goat IgG-TR: sc-2783 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

## RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor Rab 6A, Rab 6B and Rab 6C gene expression knockdown using RT-PCR Primer: Rab 6 (h)-PR: sc-44063-PR (20  $\mu$ l). Annealing temperature for the primers should be  $55-60^{\circ}$  C and the extension temperature should be  $68-72^{\circ}$  C.

## BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

## RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.

## PROTOCOLS

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