

Rab 33A siRNA (m): sc-152637

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 33B and Rab 33A share conserved effector domains, but share only 55.3% overall amino acid identity. This suggests that Rab 33B and Rab 33A may interact with similar effector and regulatory proteins. Rab 33B is a ubiquitously expressed member of the Rab family of proteins and colocalizes with α -mannosidase II (a Golgi marker) at the medial-Golgi cisternae. Rab 33B is believed to participate in intra-Golgi transport. Rab 33A is predominantly expressed in CD8⁺ T cells and is involved in tuberculosis (TB) processes.

REFERENCES

1. Zheng, J.Y., et al. 1998. A novel Rab GTPase, Rab33B, is ubiquitously expressed and localized to the medial Golgi cisternae. *J. Cell Sci.* 111: 1061-1069.
2. Valsdottir, R., et al. 2001. Identification of rabaptin-5, rabex-5, and GM130 as putative effectors of rab33b, a regulator of retrograde traffic between the Golgi apparatus and ER. *FEBS Lett.* 508: 201-209.
3. Online Mendelian Inheritance in Man, OMIM™. 2002. Johns Hopkins University, Baltimore, MD. MIM Number: 605950. World Wide Web URL: <http://www.ncbi.nlm.nih.gov/omim/>
4. Junutula, J.R., et al. 2004. Rab14 is involved in membrane trafficking between the Golgi complex and endosomes. *Mol. Biol. Cell* 15: 2218-2229.
5. Jiang, S. and Storrie, B. 2005. Cisternal rab proteins regulate Golgi apparatus redistribution in response to hypotonic stress. *Mol. Biol. Cell* 16: 2586-2596.
6. Jacobsen, M., et al. 2005. Ras-associated small GTPase 33A, a novel T cell factor, is down-regulated in patients with tuberculosis. *J. Infect. Dis.* 192: 1211-1218.

CHROMOSOMAL LOCATION

Genetic locus: Rab33a (mouse) mapping to X A4.

PRODUCT

Rab 33A siRNA (m) 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 Rab 33A shRNA Plasmid (m): sc-152637-SH and Rab 33A shRNA (m) Lentiviral Particles: sc-152637-V as alternate gene silencing products.

For independent verification of Rab 33A (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-152637A, sc-152637B and sc-152637C.

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 33A siRNA (m) is recommended for the inhibition of Rab 33A expression in mouse 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 33A/B (D-12): sc-271199 is recommended as a control antibody for monitoring of Rab 33A 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 33A gene expression knockdown using RT-PCR Primer: Rab 33A (m)-PR: sc-152637-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.