

Rab 45 siRNA (m): sc-152649

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 45, also known as RASEF (RAS and EF-hand domain containing protein), is a member of the Rab family of proteins that contains two EF-hand domains, a coiled coil motif and one C-terminal Rab domain. Localizing to the perinuclear region of the cytoplasm, Rab 45 can form a homodimer and binds both GDP and GTP. Rab 45 expression is down-regulated in cutaneous melanoma cells, suggesting a possible role for Rab 45 as a tumor suppressor.

REFERENCES

1. Online Mendelian Inheritance in Man, OMIM™. 2002. Johns Hopkins University, Baltimore, MD. MIM Number: 611344. World Wide Web URL: <http://www.ncbi.nlm.nih.gov/omim/>
2. Sweetser, D.A., et al. 2005. Delineation of the minimal commonly deleted segment and identification of candidate tumor-suppressor genes in del(9q) acute myeloid leukemia. *Genes Chromosomes Cancer* 44: 279-291.
3. Jönsson, G., et al. 2005. Mapping of a novel ocular and cutaneous malignant melanoma susceptibility locus to chromosome 9q21.32. *J. Natl. Cancer Inst.* 97: 1377-1382.
4. Kokkinakis, D.M., et al. 2006. Mitotic arrest, apoptosis, and sensitization to chemotherapy of melanomas by methionine deprivation stress. *Mol. Cancer Res.* 4: 575-589.
5. Shintani, M., et al. 2007. Characterization of Rab45/RASEF containing EF-hand domain and a coiled-coil motif as a self-associating GTPase. *Biochem. Biophys. Res. Commun.* 357: 661-667.
6. Maat, W., et al. 2008. Epigenetic regulation identifies RASEF as a tumor-suppressor gene in uveal melanoma. *Invest. Ophthalmol. Vis. Sci.* 49: 1291-1298.

CHROMOSOMAL LOCATION

Genetic locus: Rasef (mouse) mapping to 4 C3.

PRODUCT

Rab 45 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 45 shRNA Plasmid (m): sc-152649-SH and Rab 45 shRNA (m) Lentiviral Particles: sc-152649-V as alternate gene silencing products.

For independent verification of Rab 45 (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-152649A, sc-152649B and sc-152649C.

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 45 siRNA (m) is recommended for the inhibition of Rab 45 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 45 (SJ-12): sc-81925 is recommended as a control antibody for monitoring of Rab 45 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 45 gene expression knockdown using RT-PCR Primer: Rab 45 (m)-PR: sc-152649-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.