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SANTA CRUZ BIOTECHNOLOGY, INC.

Rab 27a siRNA (h): sc-41834

BACKGROUND

The Rab family of low molecular weight GTPases are critical regulators of vesicular transport. Rab proteins cycle between an active GTP-bound state, which recruits specific effector proteins, and an inactive GDP-bound state. Two members of this family, Rab 27a and Rab 27b, have overlapping functions, but differ in tissue specificity. Rab 27a is widely expressed with significant expression in pancreatic islets and pituitary tissue, and low expression in brain. Rab 27b is also expressed in pituitary tissue, but is more significantly expressed in brain and spleen. Rab 27a regulates diverse processes involving lysosome-related organelles, including melanosome motility in melanocytes and lytic granule release in cytotoxic T lymphocytes. Mutations in the Rab 27a gene result in Griscelli syndrome (GS) or the corresponding mouse model ashen, a rare autosomal recessive disorder characterized by hypopigmentation, prolonged bleeding times, and platelet storage pool deficiency. In GS, Rab 27a is not available to mediate the recruitment of melanosomes via the Actin motor, Myosin Va. The human Rab 27b gene maps to chromosome 18q21.1, and encodes a protein that is involved in pituitary hormone secretion. Rab 27b may be functionally redundant to Rab 27a, as it can rescue Rab 27a mutants.

REFERENCES

1. Ramalho, J.S., et al. 2001. Chromosomal mapping, gene structure and characterization of the human and murine Rab 27b gene. BMC Genet. 2: 2.
2. Barral, D.C., et al. 2002. Functional redundancy of Rab 27 proteins and the pathogenesis of Griscelli syndrome. J. Clin. Invest. 110: 247-257.
3. Zhao, S., et al. 2002. Involvement of Rab 27b in the regulated secretion of pituitary hormones. Endocrinology 143: 1817-1824.

CHROMOSOMAL LOCATION

Genetic locus: RAB27A (human) mapping to 15q21.3.

PRODUCT

Rab 27a siRNA (h) 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Rab 27a shRNA Plasmid (h): sc-41834-SH and Rab 27a shRNA (h) Lentiviral Particles: sc-41834-V as alternate gene silencing products.

For independent verification of Rab 27a (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-41834A, sc-41834B and sc-41834C.

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 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

Rab 27a siRNA (h) is recommended for the inhibition of Rab 27a 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 µM in 66 µl. 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 27a (E-8): sc-74586 is recommended as a control antibody for monitoring of Rab 27a 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-IgGκ BP-HRP: sc-516102 or m-IgGκ 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-IgGκ BP-FITC: sc-516140 or m-IgGκ 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 27a gene expression knockdown using RT-PCR Primer: Rab 27a (h)-PR: sc-41834-PR (20 µl, 429 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Saxena, S.K., et al. 2006. Rab 27a regulates epithelial sodium channel (ENaC) activity through Synaptotagmin-like protein (Slp5) and Munc13-4 effector mechanism. Biochem. Biophys. Res. Commun. 344: 651-657.
2. Saxena, S.K., et al. 2006. Rab27a negatively regulates CFTR chloride channel function in colonic epithelia: involvement of the effector proteins in the regulatory mechanism. Biochem. Biophys. Res. Commun. 346: 259-267.

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.