

Rab 3 GAP p130 siRNA (h): sc-94704

BACKGROUND

Rab3 proteins are involved in regulated exocytosis of neurotransmitters and hormones. Rab 3 GAP p130, also known as Rab3 GTPase-activating protein catalytic subunit, is a 981 amino acid protein that belongs to the Rab3-GAP catalytic subunit family. Rab 3 GAP p130 converts active RAB3-GTP to the inactive form RAB3-GDP, and is required for normal eye and brain development. Defects in Rab 3 GAP p130 are the cause of Warburg micro syndrome 1 (WARBM1). WARBM1 is a severe autosomal recessive disorder characterized by developmental abnormalities of the eye and central nervous system and by microgenitalia. The Rab 3 GAP p130 protein may participate in neurodevelopmental processes such as proliferation, migration and differentiation before synapse formation, and non-synaptic vesicular release of neurotransmitters. Existing as two alternatively spliced isoforms, the Rab 3 GAP p130 gene is conserved in chimpanzee, canine, bovine, mouse, chicken, zebrafish and fruit fly, and maps to human chromosome 2q21.3.

REFERENCES

1. Fukui, K., et al. 1997. Isolation and characterization of a GTPase activating protein specific for the Rab3 subfamily of small G proteins. *J. Biol. Chem.* 272: 4655-4658.
2. Oishi, H., et al. 1998. Localization of the Rab3 small G protein regulators in nerve terminals and their involvement in Ca^{2+} -dependent exocytosis. *J. Biol. Chem.* 273: 34580-34585.
3. Online Mendelian Inheritance in Man, OMIM™. 1998. Johns Hopkins University, Baltimore, MD. MIM Number: 602536. World Wide Web URL: <http://www.ncbi.nlm.nih.gov/omim/>
4. Clabecq, A., et al. 2000. Biochemical characterization of Rab3-GTPase-activating protein reveals a mechanism similar to that of Ras-GAP. *J. Biol. Chem.* 275: 31786-31791.
5. Aligianis, I.A., et al. 2005. Mutations of the catalytic subunit of RAB3GAP cause Warburg Micro syndrome. *Nat. Genet.* 37: 221-223.
6. Sakane, A., et al. 2006. Rab3 GTPase-activating protein regulates synaptic transmission and plasticity through the inactivation of Rab3. *Proc. Natl. Acad. Sci. USA* 103: 10029-10034.

CHROMOSOMAL LOCATION

Genetic locus: RAB3GAP1 (human) mapping to 2q21.3.

PRODUCT

Rab 3 GAP p130 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Rab 3 GAP p130 shRNA Plasmid (h): sc-94704-SH and Rab 3 GAP p130 shRNA (h) Lentiviral Particles: sc-94704-V as alternate gene silencing products.

For independent verification of Rab 3 GAP p130 (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-94704A, sc-94704B and sc-94704C.

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 3 GAP p130 siRNA (h) is recommended for the inhibition of Rab 3 GAP p130 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 3 GAP p130 (G-3): sc-393540 is recommended as a control antibody for monitoring of Rab 3 GAP p130 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 3 GAP p130 gene expression knockdown using RT-PCR Primer: Rab 3 GAP p130 (h)-PR: sc-94704-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.