

GARNL4 siRNA (r): sc-270421

BACKGROUND

The Rap family of small GTPases is closely related to Ras and may function as an antagonist to the Ras signaling pathway by trapping Ras effectors in an inactive complex. Similar to other guanine-binding proteins (such as the heterotrimeric G proteins), the Ras proteins cycle between an active guanosine-triphosphate (GTP) bound form and an inactive guanosine-diphosphate (GDP) bound form. The weak intrinsic GTPase activity of Ras proteins is greatly enhanced by the action of GTPase-activating proteins (GAPs). GARNL4 (GTPase-activating Rap/Ran-GAP domain-like protein 4), also known as RAP1GA3 or RAP1GAP2, is a 730 amino acid cytoplasmic and perinuclear protein that contains one Rap-GAP domain. GARNL4 is considered a GTPase activator for the nuclear Ras-related regulatory protein Rap 1A, converting it to the putatively inactive GDP-bound state. Existing as three alternatively spliced isoforms, GARNL4 is expressed in lymphocytes, heart, testis and pancreas. Isoforms 1 and 2 are expressed in platelets with isoform 2 being the predominant form.

REFERENCES

1. Bos, J.L. 1998. All in the family? New insights and questions regarding interconnectivity of Ras, Rap1 and Ral. *EMBO J.* 17: 6776-6782.
2. Zwartkruis, F.J. and Bos, J.L. 1999. Ras and Rap1: two highly related small GTPases with distinct function. *Exp. Cell Res.* 253: 157-165.
3. Tsukamoto, N., Hattori, M., Yang, H., Bos, J.L. and Minato, N. 1999. Rap1 GTPase-activating protein SPA-1 negatively regulates cell adhesion. *J. Biol. Chem.* 274: 18463-18469.
4. Archelos, J.J. and Hartung, H.P. 2000. Pathogenetic role of autoantibodies in neurological diseases. *Trends Neurosci.* 23: 317-327.
5. Ishida, D., Yang, H., Masuda, K., Uesugi, K., Kawamoto, H., Hattori, M. and Minato, N. 2003. Antigen-driven T cell anergy and defective memory T cell response via deregulated Rap1 activation in SPA-1-deficient mice. *Proc. Natl. Acad. Sci. USA* 100: 10919-10924.
6. Schultess, J., Danielewski, O. and Smolenski, A.P. 2005. Rap1GAP2 is a new GTPase-activating protein of Rap1 expressed in human platelets. *Blood* 105: 3185-3192.
7. Willard, F.S., Low, A.B., McCudden, C.R. and Siderovski, D.P. 2007. Differential G_{α} interaction capacities of the GoLoco motifs in Rap GTPase activating proteins. *Cell. Signal.* 19: 428-438.
8. Hoffmeister, M., Riha, P., Neumüller, O., Danielewski, O., Schultess, J. and Smolenski, A.P. 2008. Cyclic nucleotide-dependent protein kinases inhibit binding of 14-3-3 to the GTPase-activating protein Rap1GAP2 in platelets. *J. Biol. Chem.* 283: 2297-2306.
9. Neumüller, O., Hoffmeister, M., Babica, J., Prella, C., Gegenbauer, K. and Smolenski, A.P. 2009. Synaptotagmin-like protein 1 interacts with the GTPase-activating protein Rap1GAP2 and regulates dense granule secretion in platelets. *Blood* 114: 1396-1404.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.

CHROMOSOMAL LOCATION

Genetic locus: Rap1gap2 (rat) mapping to 10q24.

PRODUCT

GARNL4 siRNA (r) 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 GARNL4 shRNA Plasmid (r): sc-270421-SH and GARNL4 shRNA (r) Lentiviral Particles: sc-270421-V as alternate gene silencing products.

For independent verification of GARNL4 (r) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-270421A, sc-270421B and sc-270421C.

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

GARNL4 siRNA (r) is recommended for the inhibition of GARNL4 expression in rat 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.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor GARNL4 gene expression knockdown using RT-PCR Primer: GARNL4 (r)-PR: sc-270421-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.