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Rho GAP p190-B siRNA (h): sc-44077

BACKGROUND

Rho GAP p190-B (ARHGAP5, RhoGAP5; Rho GTPase activating protein 5, p190-B, RasGAP-associated p105 protein) negatively regulates Rho GTPases by stimulating the hydrolysis of bound GTP. Two transcript variants encoding different isoforms have been found for the human gene. Rho GAP p190-B localizes diffusely in the cytoplasm, and in fibrillar patterns that co-localize with $\alpha5/\beta1$ Integrin receptor in normal human foreskin fibroblasts, RD muscle cells and HT-1080 cells. Rho GAP p190-B interacts with transmembrane plexin receptors and mediates semaphorin signalling to the Actin cytoskeleton, guiding cell migration and axon extension. Plexin activation leads to the disassembly of integrin-based focal adhesive structures and to Actin cytoskeleton remodelling and inhibition of cell migration. SHP-2-dependent dephosphorylation of Rho GAP p190-B leads to the activation of RhoA in myoblasts, supporting the idea that myogenesis is under the influence of SHP-2 activity on the Rho GAP p190/RhoA pathway.

REFERENCES

1. Foster, R., et al. 1994. p190 RhoGAP, the major RasGAP-associated protein, binds GTP directly. *Mol. Cell. Biol.* 14: 7173-7181.
2. Chang, J.H., et al. 1995. c-Src regulates the simultaneous rearrangement of Actin cytoskeleton, p190RhoGAP, and p120RasGAP following epidermal growth factor stimulation. *J. Cell Biol.* 130: 355-368.
3. Burbelo, P.D., et al. 1995. p190-B, a new member of the Rho GAP family, and Rho are induced to cluster after integrin cross-linking. *J. Biol. Chem.* 270: 30919-30926.

CHROMOSOMAL LOCATION

Genetic locus: ARHGAP5 (human) mapping to 14q12.

PRODUCT

Rho GAP p190-B 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 Rho GAP p190-B shRNA Plasmid (h): sc-44077-SH and Rho GAP p190-B shRNA (h) Lentiviral Particles: sc-44077-V as alternate gene silencing products.

For independent verification of Rho GAP p190-B (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-44077A, sc-44077B and sc-44077C.

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

Rho GAP p190-B siRNA (h) is recommended for the inhibition of Rho GAP p190-B 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

Rho GAP p190-B (G-11): sc-393241 is recommended as a control antibody for monitoring of Rho GAP p190-B 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 Rho GAP p190-B gene expression knockdown using RT-PCR Primer: Rho GAP p190-B (h)-PR: sc-44077-PR (20 μ l, 455 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Daubon, T., et al. 2016. VEGF-A stimulates podosome-mediated collagen-IV proteolysis in microvascular endothelial cells. *J. Cell Sci.* 129: 2586-2598.
2. Barabutis, N., et al. 2018. Wild-type p53 enhances endothelial barrier function by mediating RAC1 signalling and RhoA inhibition. *J. Cell. Mol. Med.* 22: 1792-1804.

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.