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Rho GAP p190B siRNA (m): sc-44986

BACKGROUND

Rho GAP p190, also designated ARHGAP5, Rho GTPase activating protein 5 (Rho GAP 5), p190-B and Ras GAP-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 localizes diffusely in the cytoplasm, and in fibrillar patterns that co-localize with $\alpha 5/\beta 1$ Integrin receptor in normal human foreskin fibroblasts, RD muscle cells and HT-1080 cells. Rho GAP p190 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 leads to the activation of Rho A in myoblasts, supporting the idea that myogenesis is under the influence of SHP-2 activity on the Rho GAP p190/Rho A pathway.

REFERENCES

1. Foster, R., et al. 1994. Rho GAP p190, the major Ras GAP-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, Rho GAP p190, and Ras GAP p120 following epidermal growth factor stimulation. *J. Cell Biol.* 130: 355-368.
3. Burbelo, P.D., et al. 1995. p190B, a new member of the Rho GAP family, and Rho are induced to cluster after Integrin cross-linking. *J. Biol. Chem.* 270: 30919-30926.
4. Tatsis, N., et al. 1998. The function of the p190 Rho GTPase-activating protein is controlled by its N-terminal GTP binding domain. *J. Biol. Chem.* 273: 34631-34638.
5. Chen, J.C., et al. 2003. Oncogenic Ras leads to Rho activation by activating the mitogen-activated protein kinase pathway and decreasing Rho GTPase-activating protein activity. *J. Biol. Chem.* 278: 2807-2818.

CHROMOSOMAL LOCATION

Genetic locus: Arhgap5 (mouse) mapping to 12 C1.

PRODUCT

Rho GAP p190-B 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 Rho GAP p190-B shRNA Plasmid (m): sc-44986-SH and Rho GAP p190-B shRNA (m) Lentiviral Particles: sc-44986-V as alternate gene silencing products.

For independent verification of Rho GAP p190-B (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-44986A, sc-44986B and sc-44986C.

PROTOCOLS

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

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

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 (m)-PR: sc-44986-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.