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Ral B siRNA (m): sc-41845

BACKGROUND

Ral A and Ral B constitute a distinct subfamily of Ras-related GTPases (i.e., GDP/GTP binding proteins). Ral proteins are activated by a unique nucleotide exchange factor, Ral GDS, and deactivated by a distinct GTPase-activating protein. Unlike Ras proteins, Ral A and Ral B fail to induce transformed foci when activated variants are expressed in various recipient cells. A potential downstream target of Ral, designated Ral BP-1, has been shown to contain a Rho-GTPase-activating domain. This Rho-GTPase-activating domain interacts preferentially with the Rho family member Cdc42. A Ras/Ral signaling pathway has been reported to mediate phospholipase D (PLD) activation by v-Src, thus indicating PLD as another downstream target of Ral A.

REFERENCES

1. Wildey, G.M., et al. 1993. Isolation of cDNA clones and tissue expression of rat ral A and ral B GTP-binding proteins. *Biochem. Biophys. Res. Commun.* 194: 552-559.
2. Spaargaren, M., et al. 1994. Identification of the guanine nucleotide dissociation stimulator for Ral as a putative effector molecule of R-Ras, H-Ras, K-Ras, and Rap. *Proc. Natl. Acad. Sci. USA* 91: 12609-12613.
3. Cantor, S.B., et al. 1995. Identification and characterization of Ral-binding protein 1, a potential downstream target of Ral GTPases. *Mol. Cell. Biol.* 15: 4578-4584.
4. Jullien-Flores, V., et al. 1995. Bridging Ral GTPase to Rho pathways. RLP176, a Ral effector with CDC42/Rac GTPase-activating protein activity. *J. Biol. Chem.* 270: 22473-22477.
5. Jiang, H., et al. 1995. Involvement of Ral GTPase in v-Src-induced phospholipase D activation. *Nature* 378: 409-412.

CHROMOSOMAL LOCATION

Genetic locus: Ralb (mouse) mapping to 1 E2.3.

PRODUCT

Ral 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 Ral B shRNA Plasmid (m): sc-41845-SH and Ral B shRNA (m) Lentiviral Particles: sc-41845-V as alternate gene silencing products.

For independent verification of Ral 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-41845A, sc-41845B and sc-41845C.

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.

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

Ral B siRNA (m) is recommended for the inhibition of Ral 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

Ral B (C-8): sc-390108 is recommended as a control antibody for monitoring of Ral 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 Ral B gene expression knockdown using RT-PCR Primer: Ral B (m)-PR: sc-41845-PR (20 μ l, 585 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.