

Ral A siRNA (h): sc-41842

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. Hofer, F., et al. 1994. Activated Ras interacts with the Ral guanine nucleotide dissociation stimulator. *Proc. Natl. Acad. Sci. USA* 91: 11089-11093.
3. Spaargaren, M. and Bischoff, J.R. 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.
4. Jiang, H., et al. 1995. Involvement of Ral GTPase in v-Src-induced phospholipase D activation. *Nature* 378: 409-412.
5. 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.
6. Jullien-Flores, V., et al. 1995. Bridging Ral GTPase to Rho pathways. RLIP76, a Ral effector with Cdc42/Rac GTPase-activating protein activity. *J. Biol. Chem.* 270: 22473-22477.

CHROMOSOMAL LOCATION

Genetic locus: RALA (human) mapping to 7p14.1.

PRODUCT

Ral A siRNA (h) is a target-specific 19-25 nt siRNA 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 A shRNA Plasmid (h): sc-41842-SH and Ral A shRNA (h) Lentiviral Particles: sc-41842-V as alternate gene silencing 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 A siRNA (h) is recommended for the inhibition of Ral A 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

Ral A (F-5): sc-374462 is recommended as a control antibody for monitoring of Ral A 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 A gene expression knockdown using RT-PCR Primer: Ral A (h)-PR: sc-41842-PR (20 μ l, 568 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Tecleab, A., et al. 2014. Ral GTPase down-regulation stabilizes and reactivates p53 to inhibit malignant transformation. *J. Biol. Chem.* 289: 31296-31309.
2. Padavano, J., et al. 2015. Mutant K-Ras promotes invasion and metastasis in pancreatic cancer through GTPase signaling pathways. *Cancer Growth Metastasis* 8: 95-113.

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.