

Rad GTPase siRNA (h): sc-61433

BACKGROUND

The Ras-encoded family of proteins bind GDP and GTP with high affinity. They possess a low level of intrinsic GTPase activity that increases more than 100-fold when interacting with cytosolic GTPase activating protein (GAP). Ras family members include H-Ras, K-Ras, N-Ras, M-Ras, R-Ras, E-Ras, Rheb, TC 21, RASL11B, and Rad (Ras associated with diabetes) GTPase. Rad GTPase is a GTP-binding protein that is similar to Ras but has unique features. Unlike other small GTPases, Rad GTPase lacks typical prenylation motifs at its C terminus. The Rad GTPase enzyme binds calmodulin, inhibits vascular lesion formation, has low intrinsic GTPase activity and cannot be stimulated by any known GAP molecules. Rad GTPase is expressed in skeletal muscle, cardiac muscle and lung tissues and is overexpressed in the skeletal muscle tissue of individuals with type II diabetes. It is also expressed to a lesser extent in the placenta, adipose tissue and kidney.

REFERENCES

1. Zhu, J., Reynet, C., Caldwell, J.S. and Kahn, C.R. 1995. Characterization of Rad, a new member of Ras/GTPase superfamily, and its regulation by a unique GTPase-activating protein (GAP)-like activity. *J. Biol. Chem.* 270: 4805-4812.
2. Zhu, J., Tseng, Y.H., Kantor, J.D., Rhodes, C.J., Zetter, B.R., Moyers, J.S. and Kahn, C.R. 2000. Interaction of the Ras-related protein associated with diabetes Rad and the putative tumor metastasis suppressor NM23 provides a novel mechanism of GTPase regulation. *Proc. Natl. Acad. Sci. USA* 96: 14911-14918.
3. Fu, M., Zhang, J., Tseng, Y.H., Cui, T., Zhu, X., Xiao, Y., Mou, Y., De Leon, H., Chang, M.M., Hamamori, Y., Kahn, C.R. and Chen, Y.E. 2005. Rad GTPase attenuates vascular lesion formation by inhibition of vascular smooth muscle cell migration. *Circulation* 111: 1071-1077.
4. Langston, L.D. and Symington, L.S. 2005. Opposing roles for DNA structure-specific proteins Rad1, MSH2, MSH3, and Sgs1 in yeast gene targeting. *EMBO J.* 24: 2214-2223.

CHROMOSOMAL LOCATION

Genetic locus: RRAD (human) mapping to 16q22.1.

PRODUCT

Rad GTPase 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 Rad GTPase shRNA Plasmid (h): sc-61433-SH and Rad GTPase shRNA (h) Lentiviral Particles: sc-61433-V as alternate gene silencing products.

For independent verification of Rad GTPase (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-61433A, sc-61433B and sc-61433C.

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

Rad GTPase siRNA (h) is recommended for the inhibition of Rad GTPase 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

Rad GTPase (B-10): sc-373988 is recommended as a control antibody for monitoring of Rad GTPase 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 Rad GTPase gene expression knockdown using RT-PCR Primer: Rad GTPase (h)-PR: sc-61433-PR (20 μ l, 422 bp). 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.