

Rit siRNA (r): sc-270500

BACKGROUND

Neuronal activity dramatically increases the concentration of cytosolic Ca²⁺, which then serves as a second messenger to direct diverse cellular responses. Calmodulin is a primary mediator of Ca²⁺ signals in the nervous system. Ric, a protein related to the Ras subfamily of small GTPases, has the ability to bind calmodulin. In addition, two Ras-like human proteins, Rin and Rit (Ric-related gene expressed in many tissues), which are 71% and 66% identical to RIC respectively, share related G₂ domains with Ric. While most members of the Ras subfamily are plasma membrane-associated and generally require a C-terminal isoprenyl group to bind to the plasma membrane, Rit and Rin lack the recognition signal for C-terminal prenylation. Transiently expressed Rit and Rin are plasma membrane-localized because both proteins contain a C-terminal cluster of basic amino acids, which provides a mechanism for membrane association. Rin binds calmodulin through a C-terminal binding motif. Rit and Ric are widely expressed, whereas expression of Rin is restricted to the neuron system. In conclusion, Rit and Rin define a novel subfamily of Ras-related proteins.

REFERENCES

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2. Casey, P.J. 1994. Lipid modifications of G proteins. *Curr. Opin. Cell Biol.* 10: 219-225.
3. Casey, P.J. 1995. Protein lipidation in cell signaling. *Science* 268: 221-225.
4. Wes, P.D., Yu, M. and Montell, C. 1996. RIC, a calmodulin-binding Ras-like GTPase. *EMBO J.* 15: 5839-5848.
5. Lee, C.J., Della, N.G., Chew, C.E. and Zack, D.J. 1996. Rin, a neuron-specific and calmodulin-binding small G-protein, and Rit define a novel subfamily of Ras proteins. *J. Neurosci.* 16: 6784-6794.

CHROMOSOMAL LOCATION

Genetic locus: Rit1 (rat) mapping to 2q34.

PRODUCT

Rit siRNA (r) 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 μM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Rit shRNA Plasmid (r): sc-270500-SH and Rit shRNA (r) Lentiviral Particles: sc-270500-V as alternate gene silencing products.

For independent verification of Rit (r) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-270500A, sc-270500B and sc-270500C.

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 μl of the RNase-free water provided. Resuspension of the siRNA duplex in 330 μl of RNase-free water makes a 10 μM solution in a 10 μM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

Rit siRNA (r) is recommended for the inhibition of Rit expression in rat 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 μM in 66 μl. 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.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor Rit gene expression knockdown using RT-PCR Primer: Rit (r)-PR: sc-270500-PR (20 μl). 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.