SANTA CRUZ BIOTECHNOLOGY, INC.

Repac siRNA (h): sc-89700

BACKGROUND

Members of the RAS subfamily of GTPases function in signal transduction as GTP/GDP-regulated switches that cycle between inactive GDP- and active GTP-bound states. Guanine nucleotide exchange factors (GEFs), such as Repac, serve as RAS activators by promoting acquisition of GTP to maintain the active GTP-bound state and are the key link between cell surface receptors and RAS activation. Repac, also known as Rap guanine nucleotide exchange factor 5 (RAPGEF5), is a 580 amino acid protein that is expressed in brain and testis, with weaker expression in heart, placenta, lung, pancreas and small intestine. Existing as two alternatively spliced isoforms, the Repac gene is conserved in cow, mouse and rat. The gene that encodes Repac maps to human chromosome 7p15.3, with high expression of this region linking to positive response to erlotinib therapy for those with non-small-cell lung cancer.

REFERENCES

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3. de Rooij, J., et al. 2000. Mechanism of regulation of the Epac family of cAMP-dependent RapGEFs. J. Biol. Chem. 275: 20829-20836.
4. Rebhun, J.F., et al. 2000. Identification of guanine nucleotide exchange factors (GEFs) for the Rap1 GTPase. Regulation of MR-GEF by M-Ras-GTP interaction. J. Biol. Chem. 275: 34901-34908.
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CHROMOSOMAL LOCATION

Genetic locus: RAPGEF5 (human) mapping to 7p15.3.

PRODUCT

Repac 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Repac shRNA Plasmid (h): sc-89700-SH and Repac shRNA (h) Lentiviral Particles: sc-89700-V as alternate gene silencing products.

For independent verification of Repac (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-89700A, sc-89700B and sc-89700C.

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

Repac siRNA (h) is recommended for the inhibition of Repac 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 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 Repac gene expression knockdown using RT-PCR Primer: Repac (h)-PR: sc-89700-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.

PROTOCOLS

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