



Ras-GRF2 siRNA (h): sc-36395

BACKGROUND

A critical step in signal transduction responses to stimulation of cell surface receptors by their ligands involves the accumulation of Ras proteins in their active GTP-bound state. To reach their active GTP-bound state, Ras proteins must first release bound GDP, a rate limiting step mediated by a guanine nucleotide releasing factor (GRF). The mammalian Ras p21 GRF protein has been designated Ras-GRF1 p140. Ras-GRF1 accelerates release of GDP from H- and N-Ras p21 protein *in vitro*, but not from the related Ral A or Cdc42Hs GTP-binding proteins. Of interest, a region mapping within the amino terminal domain of Ras-GRF1 is similar to both the human breakpoint cluster protein, Bcr, and the Dbl proto-oncogene product, a guanine nucleotide-releasing factor for Cdc42Hs. Ras-GRF2 p135 has also been identified. Ras-GRF2 p135 is highly homologous to Ras-GRF1 p140 except in the region between the REM and CDC25 domains and appears to function similarly to Ras-GRF1 p140.

REFERENCES

1. Ron, D., et al. 1988. Molecular cloning and characterization of the human dbl proto-oncogene: evidence that its overexpression is sufficient to transform NIH/3T3 cells. *EMBO J.* 7: 2465-2473.
2. Downward, J., et al. 1990. Stimulation of p21^{ras} upon T cell activation. *Nature* 346: 719-723.
3. Gibbs, J.B., et al. 1990. Modulation of guanine nucleotides bound to Ras in NIH/3T3 cells by oncogenes, growth factors, and the GTPase activating protein (GAP). *J. Biol. Chem.* 265: 20437-20442.
4. Wolfman, A., et al. 1990. Cytosolic protein catalyzes the release of GDP from p21^{ras}. *Science* 248: 247-249.
5. Downward, J., et al. 1990. Identification of a nucleotide exchange-promoting activity for p21^{ras}. *Proc. Natl. Acad. Sci. USA* 87: 5998-6002.
6. Hart, M.J., et al. 1991. Catalysis of guanine nucleotide exchange on the Cdc42Hs protein by the Dbl oncogene product. *Nature* 354: 311-313.
7. Shou, C., et al. 1992. Molecular cloning of cDNAs encoding a guanine-nucleotide-releasing factor for Ras p21. *Nature* 358: 351-354.
8. Fam, N.P., et al. 1997. Cloning and characterization of Ras-GRF2, a novel guanine nucleotide exchange factor for Ras. *Mol. Cell. Biol.* 17: 1396-1406.

CHROMOSOMAL LOCATION

Genetic locus: RASGRF2 (human) mapping to 5q14.1.

PRODUCT

Ras-GRF2 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 Ras-GRF2 shRNA Plasmid (h): sc-36395-SH and Ras-GRF2 shRNA (h) Lentiviral Particles: sc-36395-V as alternate gene silencing products.

For independent verification of Ras-GRF2 (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-36395A, sc-36395B and sc-36395C.

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

Ras-GRF2 siRNA (h) is recommended for the inhibition of Ras-GRF2 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.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor Ras-GRF2 gene expression knockdown using RT-PCR Primer: Ras-GRF2 (h)-PR: sc-36395-PR (20 μ l, 533 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Calvo, F., et al. 2009. Structural and spatial determinants regulating TC21 activation by RasGRF family nucleotide exchange factors. *Mol. Biol. Cell* 20: 4289-4302.
2. Wang, Q., et al. 2013. Ras-guanine-nucleotide-releasing factors 1 and 2 interact with PLC γ at focal adhesions to enable IL-1-induced Ca²⁺ signalling, ERK activation and MMP-3 expression. *Biochem. J.* 449: 771-782.

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.