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Ras-GRF1 siRNA (h): sc-41732

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. Pearsall, R.S., et al. 1998. The Rasgrf1-repeat sequence (D9Ncvs53) maps between Mod1 and Rbp1 on mouse chromosome 9 and may define a putative imprinted region. *Mamm. Genome* 9: 261-262.
2. Yoon, B.J., et al. 2002. Regulation of DNA methylation of Rasgrf1. *Nat. Genet.* 30: 92-96.
3. Arozarena, I., et al. 2004. Activation of H-Ras in the endoplasmic reticulum by the Ras-GRF family guanine nucleotide exchange factors. *Mol. Cell. Biol.* 24: 1516-1530.
4. Yoon, B., et al. 2005. Rasgrf1 imprinting is regulated by a CTCF-dependent methylation-sensitive enhancer blocker. *Mol. Cell. Biol.* 25: 11184-11190.

CHROMOSOMAL LOCATION

Genetic locus: RASGRF1 (human) mapping to 15q25.1.

PRODUCT

Ras-GRF1 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-GRF1 shRNA Plasmid (h): sc-41732-SH and Ras-GRF1 shRNA (h) Lentiviral Particles: sc-41732-V as alternate gene silencing products.

For independent verification of Ras-GRF1 (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-41732A, sc-41732B and sc-41732C.

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-GRF1 siRNA (h) is recommended for the inhibition of Ras-GRF1 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

Ras-GRF1 (D-12): sc-377234 is recommended as a control antibody for monitoring of Ras-GRF1 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 Ras-GRF1 gene expression knockdown using RT-PCR Primer: Ras-GRF1 (h)-PR: sc-41732-PR (20 μ l, 515 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Wang, Q., et al. 2011. Focal adhesions and Ras are functionally and spatially integrated to mediate IL-1 activation of ERK. *FASEB J.* 25: 3448-3464.
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.