

Ras GAP siRNA (h): sc-29467

BACKGROUND

The mammalian c-H-, c-K- and N-Ras proto-oncogenes encode ubiquitously expressed proteins. p21Ras can exist in either a physiologically quiescent GDP-binding state or a GTP-binding signal-emitting state. Oncogenic p21Ras proteins are trapped in the excited signal-emitting state because the mechanism normally employed to delimit their excitation period, hydrolysis of their bound GTP to GDP, is impaired as a result of specific mutations. Interaction of p21Ras with GTPase activating protein (GAP) can increase hydrolysis of p21Ras-bound GTP by as much as 1000-fold. The product of the neurofibromatosis type 1 gene (NF1) has also been shown to exhibit p21Ras GAP activity, and proteins that stimulate the GTPase activity of three other low molecular weight GTPases, including Rho, Rab 3A and Rap 1, have also been described.

REFERENCES

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- Trahey, M., et al. 1988. Molecular cloning of two types of GAP complementary DNA from human placenta. *Science* 242: 1697-1700.
- Vogel, U.S., et al. 1988. Cloning of bovine GAP and its interaction with oncogenic Ras p21. *Nature* 335: 90-93.
- McCormick, F. 1989. ras GTPase activating protein: signal transmitter and signal terminator. *Cell* 56: 5-8.
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- Rubinfeld, B., et al. 1991. Molecular cloning of a GTPase activating protein specific for the Krev-1 protein p21^{rap1}. *Cell* 65: 1033-1042.

CHROMOSOMAL LOCATION

Genetic locus: RASA1 (human) mapping to 5q14.3.

PRODUCT

Ras GAP 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 GAP shRNA Plasmid (h): sc-29467-SH and Ras GAP shRNA (h) Lentiviral Particles: sc-29467-V as alternate gene silencing products.

For independent verification of Ras GAP (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-29467A, sc-29467B and sc-29467C.

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

Ras GAP siRNA (h) is recommended for the inhibition of Ras GAP 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 GAP (B4F8): sc-63 is recommended as a control antibody for monitoring of Ras GAP 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 GAP gene expression knockdown using RT-PCR Primer: Ras GAP (h)-PR: sc-29467-PR (20 μ l, 560 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.