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RASSF2 siRNA (h): sc-45722

BACKGROUND

Ras is a small GTP-binding protein involved in many cellular processes, including proliferation, differentiation and apoptosis. Ras transmits signals of cell surface receptors by binding to a variety of effector molecules. In addition to the well characterized effectors Raf and PI-3 kinase, Ras also interacts with a group of homologous, noncatalytic proteins composed of RASSF1, RASSF2, RASSF3, AD037 and Nore1. RASSF1 is a potential tumor suppressor gene that plays an important role in tumor pathogenesis. Nore1 binds to Ras in response to EGF or serum stimulation, but its function has yet to be determined. RASSF2 is a nuclear protein containing a Ras-associating domain and a Sarah domain. RASSF2 isoform A is inactivated in colorectal cancer cells by CpG island promoter hypermethylation.

REFERENCES

1. Eckfeld, K., et al. 2004. RASSF4/AD037 is a potential Ras effector/tumor suppressor of the RASSF family. *Cancer Res.* 64: 8688-8693.
2. Levy, P., et al. 2004. Molecular profiling of malignant peripheral nerve sheath tumors associated with neurofibromatosis type 1, based on large-scale real-time RT-PCR. *Mol. Cancer* 3: 20.
3. Hesson, L.B., et al. 2005. CpG island promoter hypermethylation of a novel Ras-effector gene RASSF2A is an early event in colon carcinogenesis and correlates inversely with K-Ras mutations. *Oncogene* 24: 3987-3994.
4. Lambros, M.B., et al. 2005. Analysis of ovarian cancer cell lines using array-based comparative genomic hybridization. *J. Pathol.* 205: 29-40.
5. <http://harvester.embl.de/harvester/P507/P50749.htm>
6. SWISS-PROT/TrEMBL (P50749). World Wide Web URL: <http://www.expasy.ch/sprot/sprot-top.html>.

CHROMOSOMAL LOCATION

Genetic locus: RASSF2 (human) mapping to 20p13.

PRODUCT

RASSF2 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 RASSF2 shRNA Plasmid (h): sc-45722-SH and RASSF2 shRNA (h) Lentiviral Particles: sc-45722-V as alternate gene silencing products.

For independent verification of RASSF2 (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-45722A, sc-45722B and sc-45722C.

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.

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

RASSF2 siRNA (h) is recommended for the inhibition of RASSF2 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

RASSF2 (E-11): sc-376347 is recommended as a control antibody for monitoring of RASSF2 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 RASSF2 gene expression knockdown using RT-PCR Primer: RASSF2 (h)-PR: sc-45722-PR (20 μ l, 568 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.