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RASSF1 siRNA (h): sc-44070

BACKGROUND

Activated Ras proteins may induce senescence, apoptosis and terminal differentiation, though they are often associated with stimulating growth and transformation. The Ras association domain family 1 (RASSF1) gene is located at the human lung tumor suppressor locus 3p21.31 that consists of two major alternative transcripts, RASSF1A and RASSF1C. RASSF1 binds Ras in a GTP-dependent manner, both *in vivo* and *in vitro*. Activated Ras enhances and dominant negative Ras inhibits cell death induced by transient transfection of RASSF1 into 293-T cells, suggesting that RASSF1 tumor suppressor may serve as a Ras effector that mediates the apoptotic effects of oncogenic Ras. RASSF1A undergoes epigenetic inactivation in lung and breast cancers through hypermethylation of the CpG island of its promoter region. Mutant RASSF1A has significantly reduced growth suppression activity. Thus, RASSF1A is a potential tumor suppressor gene that plays an important role in a variety of tumor pathogenesis.

REFERENCES

1. Vos, M.D., et al. 2000. Ras uses the novel tumor suppressor RASSF1 as an effector to mediate apoptosis. *J. Biol. Chem.* 275: 35669-35672.
2. Damann, R., et al. 2000. Epigenetic inactivation of a RAS association domain family protein from the lung tumour suppressor locus 3p21.3. *Nat. Genet.* 25: 315-319.
3. Burbee, D.G., et al. 2001. Epigenetic activation of RASSF1A in lung and breast cancers and malignant phenotype suppression. *J. Natl. Cancer Inst.* 93: 691-699.

CHROMOSOMAL LOCATION

Genetic locus: RASSF1 (human) mapping to 3p21.31.

PRODUCT

RASSF1 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 RASSF1 shRNA Plasmid (h): sc-44070-SH and RASSF1 shRNA (h) Lentiviral Particles: sc-44070-V as alternate gene silencing products.

For independent verification of RASSF1 (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-44070A, sc-44070B and sc-44070C.

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

RASSF1 siRNA (h) is recommended for the inhibition of RASSF1 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

RASSF1 (3F3): sc-58470 is recommended as a control antibody for monitoring of RASSF1 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 RASSF1 gene expression knockdown using RT-PCR Primer: RASSF1 (h)-PR: sc-44070-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Kim, S.M., et al. 2016. RhBMP-2 activates hippo signaling through RASSF1 in esophageal cancer cells. *Sci. Rep.* 6: 26821.
2. Li, W., et al. 2018. Suppressor of hepatocellular carcinoma RASSF1A activates autophagy initiation and maturation. *Cell Death Differ.* 26: 1379-1395.

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.