

Rap 1A siRNA (m): sc-41853

BACKGROUND

Ras oncogenes encode GTP-binding proteins that are capable of transforming immortalized cells in culture. Two Ras-related human genes, designated RAP1A and RAP1B, encode 95% homologous proteins (namely Rap 1A and Rap 1B) that share a similar C-terminal Cys-Ali-Ali-Xaa sequence with Ras proteins and are ubiquitously expressed in mammalian tissues. The putative "effector" domain of Ras proteins, whose integrity is required for cell transformation as well as interaction with the putative effector protein GAP, is conserved in both Rap 1 proteins. Rap 1A is thought to interfere with Ras effector function by binding to Ras GAP in a GTP-dependent manner without affecting Rap 1A GTPase activity. Rap 2, another Ras-related protein, shares 60% identity with Rap 1A and exhibits a carbox- terminal CAAX motif and two upstream cysteines similar to those of the H-Ras, K-Ras and N-Ras proteins. In contrast with Rap 1A and Rap 1B, overexpression of Rap 2 does not interfere with the Ras signaling pathway.

REFERENCES

1. Pizon, V., et al. 1988. Human cDNAs Rap1 and Rap2 homologous to the *Drosophila* gene Dras3 encode proteins closely related to Ras in the "effector" region. *Oncogene* 3: 201-204.
2. Pizon, V., et al. 1988. Nucleotide sequence of a human cDNA encoding a Ras-related protein (Rap1B). *Nucleic Acids Res.* 16: 7719.
3. Culine, S., et al. 1989. Expression of the Ras-related Rap genes in human tumors. *Int. J. Cancer* 44: 990-994.
4. Kim, S., et al. 1990. Tissue and subcellular distributions of the Smg-21/ Rap 1/Krev-1 proteins which are partly distinct from those of c-Ras p21s. *Mol. Cell. Biol.* 10: 2645-2652.
5. Frech, M., et al. 1990. Inhibition of GTPase activating protein stimulation of Ras-p21 GTPase by the Krev-1 gene product. *Science* 249: 169-171.
6. Hata, Y., et al. 1990. Inhibition of the Ras p21 GTPase-activating protein-stimulated GTPase activity of c-Ha-Ras p21 by Smg p21 having the same putative effector domain as Ras p21s. *J. Biol. Chem.* 265: 7104-7107.
7. Beranger, F., et al. 1991. Association of the Ras-antagonistic Rap1/Krev-1 proteins with Golgi complex. *Proc. Natl. Acad. Sci. USA* 88: 1606-1610.

CHROMOSOMAL LOCATION

Genetic locus: Rap1a (mouse) mapping to 3 F2.2.

PRODUCT

Rap 1A siRNA (m) 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 Rap 1A shRNA Plasmid (m): sc-41853-SH and Rap 1A shRNA (m) Lentiviral Particles: sc-41853-V as alternate gene silencing products.

For independent verification of Rap 1A (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-41853A, sc-41853B and sc-41853C.

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

Rap 1A siRNA (m) is recommended for the inhibition of Rap 1A expression in mouse 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

Rap 1A (C-10): sc-373968 is recommended as a control antibody for monitoring of Rap 1A 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 MarkerTM 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 Rap 1A gene expression knockdown using RT-PCR Primer: Rap 1A (m)-PR: sc-41853-PR (20 μ l, 466 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.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.