

Rab 32 siRNA (h): sc-95461

BACKGROUND

Small GTP-binding proteins of the RAB family play essential roles in vesicle and granule targeting. Rab 32 is a 225 amino acid protein that belongs to the small GTPase superfamily and the Rab family. Rab 32 has an unusual GTP-binding sequence, DIAGQE, in place of the more common DTAGQE. Rab 32 acts as an A-kinase anchoring protein by binding to the type II regulatory subunit of protein kinase A and anchoring it to mitochondria. Also involved in synchronization of mitochondrial fission, Rab 32 is widely expressed with high levels in heart, liver, kidney, bone marrow, testis, colon and fetal lung. Rab 32 has been found to be frequently hypermethylated in microsatellite instability-high (MSI-H) colon cancers. Although Rab 32 methylation is rare in endometrial cancers, it is strongly associated with hMLH1 hypermethylation and MSI in gastric adenocarcinomas. The Rab 32 gene is conserved in chimpanzee, bovine, mouse, rat, chicken, zebrafish, fruit fly, mosquito and *C. elegans*, and maps to human chromosome 6q24.3.

REFERENCES

1. Bao, X., et al. 2002. Molecular cloning, bacterial expression and properties of Rab 31 and Rab 32. *Eur. J. Biochem.* 269: 259-271.
2. Alto, N.M., et al. 2002. Rab 32 is an A-kinase anchoring protein and participates in mitochondrial dynamics. *J. Cell Biol.* 158: 659-668.
3. Pereira-Leal, J.B., et al. 2003. Structural determinants of Rab and Rab escort protein interaction: Rab family motifs define a conserved binding surface. *Biochem. Biophys. Res. Commun.* 301: 92-97.
4. Mungall, A.J., et al. 2003. The DNA sequence and analysis of human chromosome 6. *Nature* 425: 805-811.
5. Alto, N.M., et al. 2003. Bioinformatic design of A-kinase anchoring protein in silico: a potent and selective peptide antagonist of type II protein kinase A anchoring. *Proc. Natl. Acad. Sci. USA* 100: 4445-4450.
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7. Hirota, Y. and Tanaka, Y. 2009. A small GTPase, human Rab 32, is required for the formation of autophagic vacuoles under basal conditions. *Cell. Mol. Life Sci.* 66: 2913-2932.

CHROMOSOMAL LOCATION

Genetic locus: RAB32 (human) mapping to 6q24.3.

PRODUCT

Rab 32 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 Rab 32 shRNA Plasmid (h): sc-95461-SH and Rab 32 shRNA (h) Lentiviral Particles: sc-95461-V as alternate gene silencing products.

For independent verification of Rab 32 (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-95461A, sc-95461B and sc-95461C.

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

Rab 32 siRNA (h) is recommended for the inhibition of Rab 32 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

Rab 32 (D-11): sc-377472 is recommended as a control antibody for monitoring of Rab 32 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 Rab 32 gene expression knockdown using RT-PCR Primer: Rab 32 (h)-PR: sc-95461-PR (20 μ l). 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.