

Rab 35 siRNA (h): sc-95797

BACKGROUND

The Ras-related superfamily of guanine nucleotide binding proteins includes the R-Ras, Rap, Ral/Rec and Rho/Rab subfamilies, all of which are thought to play an important role in either endocytosis or in biosynthetic protein transport. The process of transporting newly synthesized proteins from the endoplasmic reticulum (ER) to various stacks of the Golgi complex and to secretory vesicles involves the movement of carrier vesicles and requires Rab protein function. Rab 35, also known as Rab 1C or RAY, is a 201 amino acid lipid-anchored protein that is thought to function as a GTPase, playing a role in exocytotic and endocytotic pathways and functioning as an important factor in cytokinesis. Rab 35 is expressed ubiquitously and is encoded by a gene that maps to human chromosome 12, which houses over 1,100 genes and comprises approximately 4.5% of the human genome.

REFERENCES

1. Zhu, A.X., et al. 1994. Molecular cloning of two small GTP-binding proteins from human skeletal muscle. *Biochem. Biophys. Res. Commun.* 205: 1875-1882.
2. Zhao, H., et al. 2002. Intracellular membrane trafficking pathways in bone-resorbing osteoclasts revealed by cloning and subcellular localization studies of small GTP-binding Rab proteins. *Biochem. Biophys. Res. Commun.* 293: 1060-1065.
3. Online Mendelian Inheritance in Man, OMIM[™]. 2002. Johns Hopkins University, Baltimore, MD. MIM Number: 604199. World Wide Web URL: <http://www.ncbi.nlm.nih.gov/omim/>

CHROMOSOMAL LOCATION

Genetic locus: RAB35 (human) mapping to 12q24.23.

PRODUCT

Rab 35 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 35 shRNA Plasmid (h): sc-95797-SH and Rab 35 shRNA (h) Lentiviral Particles: sc-95797-V as alternate gene silencing products.

For independent verification of Rab 35 (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-95797A, sc-95797B and sc-95797C.

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 35 siRNA (h) is recommended for the inhibition of Rab 35 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 1 (E-8): sc-515308 is recommended as a control antibody for monitoring of Rab 35 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 35 gene expression knockdown using RT-PCR Primer: Rab 35 (h)-PR: sc-95797-PR (20 μ l, 477 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Wen, H., et al. 2021. MiR-185-5p targets RAB35 gene to regulate tumor cell-derived exosomes-mediated proliferation, migration and invasion of non-small cell lung cancer cells. *Aging* 13: 21435-21450.

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.