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SANTA CRUZ BIOTECHNOLOGY, INC.

REP-1 siRNA (h): sc-41804

BACKGROUND

Newly synthesized Rab proteins are bound to Rab escort proteins (REPs) and presented to the Rab geranylgeranyltransferase (GGTase) type II, which mediates the prenylation of Rab proteins on two carboxy terminal cysteine residues. Rab GGTase only recognizes Rab proteins as a substrate when they are bound to REP. REP remains complexed with Rab until it is transported to the appropriate subcellular membrane, although is it still unclear whether REP participates in this targeting. Two isoforms of the REP gene have been isolated, REP-1 and REP-2. The REP-1 gene, located on chromosome Xq21.2, is prone to a wide variety of mutations, including nonsense, frameshift and splice-site mutations and deletions. In patients with choroideraemia (CHM), mutations in the REP-1 gene result in progressive dystrophy of the choroid, retinal pigment epithelium and retina. CHM is an X-linked hereditary eye disease that leads to blindness later in life. REP-2 is able to bind to several Rab proteins with the same affinity as REP-1 and may act a substitute for REP-1 to prevent widespread tissue abnormalities in patients with CHM.

REFERENCES

1. Cremers, F.P., et al. 1994. REP-2, a Rab escort protein encoded by the choroideremia-like gene. J. Biol. Chem. 269: 2111-2117.
2. Alexandrov, K., et al. 1999. Characterization of the ternary complex between Rab 7, REP-1 and Rab geranylgeranyl transferase. Eur. J. Biochem. 265: 160-170.
3. Ohba, N. and Isashiki, Y. 1999. Clinical and genetic features of choroderemia. Nippon Ganka Gakkai Zasshi 103: 773-781.
4. Fujiki, K., et al. 1999. REP-1 gene mutations in Japanese patients with choroideremia. Graefes Arch. Clin. Exp. Ophthalmol. 237: 735-740.
5. Hayakawa, M., et al. 1999. Visual impairment and REP-1 gene mutations in Japanese choroideremia patients. Ophthalmic Genet. 20: 107-115.
6. Zhang, H., et al. 2000. Crystal structure of Rab geranylgeranyltransferase at 2.0 A resolution. Structure 8: 241-251.
7. Overmeyer, J.H., et al. 2001. Membrane targeting of a Rab GTPase that fails to associate with Rab escort protein (REP) or guanine nucleotide dissociationinhibitor (GDI). J. Biol. Chem. 276: 20379-2086.

CHROMOSOMAL LOCATION

Genetic locus: CHM (human) mapping to Xq21.2.

PRODUCT

REP-1 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see REP-1 shRNA Plasmid (h): sc-41804-SH and REP-1 shRNA (h) Lentiviral Particles: sc-41804-V as alternate gene silencing products.

For independent verification of REP-1 (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-41804A, sc-41804B and sc-41804C.

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 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCL, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

REP-1 siRNA (h) is recommended for the inhibition of REP-1 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 µM in 66 µl. 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

REP-1 (2F1): sc-23905 is recommended as a control antibody for monitoring of REP-1 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-IgGκ BP-HRP: sc-516102 or m-IgGκ 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-IgGκ BP-FITC: sc-516140 or m-IgGκ 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 REP-1 gene expression knockdown using RT-PCR Primer: REP-1 (h)-PR: sc-41804-PR (20 µl). 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.