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SANTA CRUZ BIOTECHNOLOGY, INC.

TBC1D8 siRNA (m): sc-154109

BACKGROUND

GTPase-activating proteins (GAPs) accelerate the intrinsic rate of GTP hydrolysis of Ras-related proteins, resulting in downregulation of their active form. TBC1D8 (TBC1 domain family member 8), also known as VRP (vascular Rab-GAP/TBC-containing protein), AD3 or HBLP1, is an 897 amino acid protein that is thought to function as a GTPase-activator for Rab proteins. TBC1D8 contains one GRAM domain and one Rab-GAP TBC domain, the latter of which is a highly conserved 200 amino acid motif that conveys the catalytic activity of GTPase-activating proteins. The gene encoding TBC1D8 maps to human chromosome 2, which houses over 1,400 genes and comprises nearly 8% of the human genome.

REFERENCES

1. Albert, S., et al. 1999. Identification of the catalytic domains and their functionally critical arginine residues of two yeast GTPase-activating proteins specific for Ypt/Rab transport GTPases. EMBO J. 18: 5216-5225.
2. Yonekura, H., et al. 1999. Antisense display—a method for functional gene screening: evaluation in a cell-free system and isolation of angiogenesis-related genes. Nucleic Acids Res. 27: 2591-2600.
3. Xu, Y.C., et al. 2002. Involvement of TRAF4 in oxidative activation of c-Jun N-terminal kinase. J. Biol. Chem. 277: 28051-28057.
4. Itoh, T., et al. 2006. Screening for target Rabs of TBC (Tre-2/Bub2/Cdc16) domain-containing proteins based on their Rab-binding activity. Genes Cells 11: 1023-1037.
5. Sklan, E.H., et al. 2007. A Rab-GAP TBC domain protein binds hepatitis C virus NS5A and mediates viral replication. J. Virol. 81: 11096-11105.
6. Ishibashi, K., et al. 2009. Identification and characterization of a novel Tre-2/Bub2/Cdc16 (TBC) protein that possesses Rab3A-GAP activity. Genes Cells 14: 41-52.

CHROMOSOMAL LOCATION

Genetic locus: Tbc1d8 (mouse) mapping to 1 B.

PRODUCT

TBC1D8 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see TBC1D8 shRNA Plasmid (m): sc-154109-SH and TBC1D8 shRNA (m) Lentiviral Particles: sc-154109-V as alternate gene silencing products.

For independent verification of TBC1D8 (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-154109A, sc-154109B and sc-154109C.

PROTOCOLS

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

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

TBC1D8 siRNA (m) is recommended for the inhibition of TBC1D8 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 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

TBC1D8 (B-5): sc-376637 is recommended as a control antibody for monitoring of TBC1D8 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 TBC1D8 gene expression knockdown using RT-PCR Primer: TBC1D8 (m)-PR: sc-154109-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.