

TBC1D20 siRNA (h): sc-76633

BACKGROUND

GTPase-activating proteins (GAPs) accelerate the intrinsic rate of GTP hydrolysis of Ras-related proteins, resulting in downregulation of their active form. TBC1D20 (TBC1 domain family, member 20), also known as FLJ45119 or C20orf140, is a 403 amino acid multi-pass membrane protein that acts as a GTPase-activating protein for Rab family members. TBC1D20 also mediates viral replication and has been identified as a host protein for hepatitis C virus nonstructural protein NS5A (HCV NS5A). HCV NS5A and TBC1D20 directly interact via the N-terminal amphipathic helix of HCV NS5A and C-terminal domain of TBC1D20. TBC1D20 contains one Rab-GAP TBC domain and exists as three isoforms which result due to alternative splicing events. The gene encoding TBC1D20 maps to human chromosome 20, which comprises approximately 2% of the human genome, contains nearly 63 million bases and encodes over 600 genes. Chromosome 20 contains a region with numerous genes which are thought important for seminal production and may be potential targets for male contraception.

REFERENCES

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2. Sklan, E.H., et al. 2007. TBC1D20 is a Rab1 GTPase-activating protein that mediates hepatitis C virus replication. *J. Biol. Chem.* 282: 36354-36361.
3. 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.
4. O'Rand, M.G., et al. 2007. Eppin: an epididymal protease inhibitor and a target for male contraception. *Soc. Reprod. Fertil. Suppl.* 63: 445-453.
5. Online Mendelian Inheritance in Man, OMIM™. 2007. Johns Hopkins University, Baltimore, MD. MIM Number: 611663. World Wide Web URL: <http://www.ncbi.nlm.nih.gov/omim/>
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CHROMOSOMAL LOCATION

Genetic locus: TBC1D20 (human) mapping to 20p13.

PRODUCT

TBC1D20 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 TBC1D20 shRNA Plasmid (h): sc-76633-SH and TBC1D20 shRNA (h) Lentiviral Particles: sc-76633-V as alternate gene silencing products.

For independent verification of TBC1D20 (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-76633A, sc-76633B and sc-76633C.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

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

TBC1D20 siRNA (h) is recommended for the inhibition of TBC1D20 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

TBC1D20 (F-9): sc-515697 is recommended as a control antibody for monitoring of TBC1D20 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 TBC1D20 gene expression knockdown using RT-PCR Primer: TBC1D20 (h)-PR: sc-76633-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

PROTOCOLS

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