

Flotillin-1 siRNA (h): sc-35391

BACKGROUND

Lipid rafts are sphingolipid- and cholesterol-rich membrane microdomains that are insoluble in nonionic detergents. Lipid rafts are important for numerous cellular processes, including signal transduction, membrane trafficking and molecular sorting. Flotillins are lipid raft components in neurons and caveolae-associated proteins in A498 kidney cells. Flotillin-1 belongs to the band 7.2/stomatin protein family, whose members are characterized by the presence of a hydrophobic N-terminal region that is predicted to form a single, outside to inside, transmembrane domain. Flotillin-1 and -2 have complementary tissue distributions and their expression levels are independently regulated. At the cellular level, Flotillin-2 is ubiquitously expressed, whereas Flotillin-1 is expressed in A498 kidney cells, muscle cell lines and fibroblasts. Flotillins form a ternary complex with CAP and Cbl, directing the localization of the CAP-Cbl complex to a lipid raft subdomain of the plasma membrane. Association of ER-X with Flotillin localizes ER-X within plasma membrane caveolae and mediates rapid oestrogen activation of the MAP kinase cascade. The expression of the Flotillins is also correlated to the progression of Alzheimer pathology.

CHROMOSOMAL LOCATION

Genetic locus: FLOT1 (human) mapping to 6p21.33.

PRODUCT

Flotillin-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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Flotillin-1 shRNA Plasmid (h): sc-35391-SH and Flotillin-1 shRNA (h) Lentiviral Particles: sc-35391-V as alternate gene silencing products.

For independent verification of Flotillin-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-35391A, sc-35391B and sc-35391C.

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

Flotillin-1 siRNA (h) is recommended for the inhibition of Flotillin-1 expression in human cells.

PROTOCOLS

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

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

Flotillin-1 (C-2): sc-74566 is recommended as a control antibody for monitoring of Flotillin-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).

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor Flotillin-1 gene expression knockdown using RT-PCR Primer: Flotillin-1 (h)-PR: sc-35391-PR (20 μ l, 494 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

- Glebov, O.O., et al. 2006. Flotillin-1 defines a clathrin-independent endocytic pathway in mammalian cells. *Nat. Cell Biol.* 8: 46-54.
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- Saallik, P., et al. 2009. Protein delivery with transportans is mediated by caveolae rather than Flotillin-dependent pathways. *Bioconjug. Chem.* 20: 877-887.
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- Raagel, H., et al. 2011. Mapping of protein transduction pathways with fluorescent microscopy. *Methods Mol. Biol.* 683: 165-179.
- Crombez, L. and Divita, G. 2011. A non-covalent peptide-based strategy for siRNA delivery. *Methods Mol. Biol.* 683: 349-360.
- Hofmann, D., et al. 2014. Mass spectrometry and imaging analysis of nanoparticle-containing vesicles provide a mechanistic insight into cellular trafficking. *ACS Nano* 8: 10077-10088.
- Xiong, Q., et al. 2019. Infection by *Anaplasma phagocytophilum* requires recruitment of low-density lipoprotein cholesterol by Flotillins. *MBio* 10 pii: e02783-18.

RESEARCH USE

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