SANTA CRUZ BIOTECHNOLOGY, INC.

Flotillin-2 siRNA (h): sc-35393

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 caveloae-associated proteins in A498 kidney cells. Flotillin-2, also designated epidermal surface antigen, is conserved in all mammalian species. 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. Stable transfection of a Flotillin-2 fusion protein in COS cells induces filopodia formation and changes epithelial cells to a neuronal appearance. 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 caveloae and mediates rapid oestrogen activation of the MAP kinase cascade. The expression of the flotillins is also correlated to the progression of Alzhemier pathology.

REFERENCES

1. Schroeder, W.T., et al. 1994. Cloning and characterization of a novel epidermal cell surface antigen (ESA). J. Biol. Chem. 269: 19983-19991.
2. Volonte, D., et al. 1999. Flotillins/cavatellins are differentially expressed in cells and tissues and form a hetero-oligomeric complex with caveloins *in vivo*. Characterization and epitope-mapping of a novel Flotillin-1 monoclonal antibody probe. J. Biol. Chem. 274: 12702-12709.
3. Hazarika, P., et al. 1999. Flotillin-2 is distinct from epidermal surface antigen (ESA) and is associated with filopodia formation. J. Cell. Biochem. 75: 147-159.
4. Baumann, C.A., et al. 2000. CAP defines a second signalling pathway required for Insulin-stimulated glucose transport. Nature 407: 202-207.
5. Toran-Allerand, C.D. 2000. Novel sites and mechanisms of oestrogen action in the brain. Novartis Found. Symp. 230: 56-69.
6. Kobubo, H., et al. 2000. Localization of flotillins in human brain and their accumulation with the progression of Alzheimer's disease pathology. Neurosci. Lett. 290: 93-96.

CHROMOSOMAL LOCATION

Genetic locus: FLOT2 (human) mapping to 17q11.2.

PRODUCT

Flotillin-2 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 Flotillin-2 shRNA Plasmid (h): sc-35393-SH and Flotillin-2 shRNA (h) Lentiviral Particles: sc-35393-V as alternate gene silencing products.

For independent verification of Flotillin-2 (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-35393A, sc-35393B and sc-35393C.

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

Flotillin-2 siRNA (h) is recommended for the inhibition of Flotillin-2 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

Flotillin-2 (B-6): sc-28320 is recommended as a control antibody for monitoring of Flotillin-2 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 Flotillin-2 gene expression knockdown using RT-PCR Primer: Flotillin-2 (h)-PR: sc-35393-PR (20 µl, 419 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Wei, S., et al. 2013. Flotillin-2 modulates Fas signaling mediated apoptosis after hyperoxia in lung epithelial cells. PLoS ONE 8: e77519.

RESEARCH USE

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