

Twinfilin-1 siRNA (m): sc-61739

BACKGROUND

Twinfilin is a highly conserved Actin monomer-binding protein that regulates cytoskeletal dynamics in organisms from yeast to mammals. Twinfilin is composed of two ADF-homology domains; it coordinates filament severing and monomer sequestering at sites of rapid Actin turnover, thus preventing assembly of the monomer into filaments. Twinfilin-1 is the mammalian homolog and is expressed in embryos and in most adult non-muscle cell types. Twinfilin-1 binds ADP-G-Actin and efficiently halts Actin filament assembly by inhibiting the nucleotide exchange on Actin monomers and directly interacting with the capping protein. Phosphatidylinositol (4,5)-bisphosphate inhibits the activity of Twinfilin-1, while two small GTPases, namely Rac1 and Cdc42, induce the redistribution of Twinfilin-1 to membrane ruffles and cell-cell contacts, respectively.

REFERENCES

- Goode, B.L., et al. 1998. Regulation of the cortical Actin cytoskeleton in budding yeast by Twinfilin, a ubiquitous Actin monomer-sequestering protein. *J. Cell Biol.* 142: 723-733.
- Vartiainen, M., et al. 2000. Mouse A6/Twinfilin is an Actin monomer-binding protein that localizes to the regions of rapid Actin dynamics. *Mol. Cell. Biol.* 20: 1772-1783.
- Palmgren, S., et al. 2001. Interactions with PIP2, ADP-Actin monomers, and capping protein regulate the activity and localization of yeast Twinfilin. *J. Cell Biol.* 155: 251-260.
- Wahlström, G., et al. 2001. Twinfilin is required for Actin-dependent developmental processes in *Drosophila*. *J. Cell Biol.* 155: 787-796.
- Palmgren, S., et al. 2002. Twinfilin, a molecular mailman for Actin monomers. *J. Cell Sci.* 115: 881-886.

CHROMOSOMAL LOCATION

Genetic locus: *Twf1* (mouse) mapping to 15 E3.

PRODUCT

Twinfilin-1 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Twinfilin-1 shRNA Plasmid (m): sc-61739-SH and Twinfilin-1 shRNA (m) Lentiviral Particles: sc-61739-V as alternate gene silencing products.

For independent verification of Twinfilin-1 (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-61739A, sc-61739B and sc-61739C.

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 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

Twinfilin-1 siRNA (m) is recommended for the inhibition of Twinfilin-1 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 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

Twinfilin-1 (E-4): sc-376539 is recommended as a control antibody for monitoring of Twinfilin-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-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 Twinfilin-1 gene expression knockdown using RT-PCR Primer: Twinfilin-1 (m)-PR: sc-61739-PR (20 μ l). 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.