

Rab11-FIP3 siRNA (m): sc-152662

BACKGROUND

The Ras superfamily of GTPases can be subdivided into the Ras, Rho/Rac, Sar, Rab, Arf, Rap and Ran subfamilies, all of which control multiple aspects of cell function, including cytoskeletal rearrangement, nuclear signaling and cell growth. Members of the Ras protein superfamily are regulated by a variety of GTPase-interaction proteins that control GTPase function. Rab11-FIP3 (Rab11 family-interacting protein 3), also known as Eferin, is a 756 amino acid GTPase-regulating protein that contains two EF-hand domains and localizes to recycling endosomes. One of several members of a family of Rab-interacting proteins, Rab11-FIP3 forms a heterooligomeric complex with Rab11-FIP4 and, once in this complex, interacts with and regulates the function of Rab 11A, Rab 11B and Rab 25. Additionally, Rab11-FIP3 is thought to play a role in vesicle docking at the midbody during cytokinesis and may be crucial for maintaining the structural integrity of the endosomal recycling compartment.

REFERENCES

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2. Hales, C.M., et al. 2001. Identification and characterization of a family of Rab11-interacting proteins. *J. Biol. Chem.* 276: 39067-39075.
3. Wallace, D.M., et al. 2002. Rab11-FIP4 interacts with Rab11 in a GTP-dependent manner and its overexpression condenses the Rab11 positive compartment in HeLa cells. *Biochem. Biophys. Res. Commun.* 299: 770-779.
4. Horgan, C.P., et al. 2004. Rab11-FIP3 localises to a Rab11-positive pericentrosomal compartment during interphase and to the cleavage furrow during cytokinesis. *Biochem. Biophys. Res. Commun.* 319: 83-94.
5. Fielding, A.B., et al. 2005. Rab11-FIP3 and FIP4 interact with Arf6 and the exocyst to control membrane traffic in cytokinesis. *EMBO J.* 24: 3389-3399.
6. Wilson, G.M., et al. 2005. The FIP3-Rab11 protein complex regulates recycling endosome targeting to the cleavage furrow during late cytokinesis. *Mol. Biol. Cell* 16: 849-860.
7. Shiba, T., et al. 2006. Structural basis for Rab11-dependent membrane recruitment of a family of Rab11-interacting protein 3 (FIP3)/Arfophilin-1. *Proc. Natl. Acad. Sci. USA* 103: 15416-15421.

CHROMOSOMAL LOCATION

Genetic locus: Rab11fip3 (mouse) mapping to 17 A3.3.

PRODUCT

Rab11-FIP3 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 Rab11-FIP3 shRNA Plasmid (m): sc-152662-SH and Rab11-FIP3 shRNA (m) Lentiviral Particles: sc-152662-V as alternate gene silencing products.

For independent verification of Rab11-FIP3 (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-152662A, sc-152662B and sc-152662C.

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

Rab11-FIP3 siRNA (m) is recommended for the inhibition of Rab11-FIP3 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

Rab11-FIP3 (6H6): sc-517043 is recommended as a control antibody for monitoring of Rab11-FIP3 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 Rab11-FIP3 gene expression knockdown using RT-PCR Primer: Rab11-FIP3 (m)-PR: sc-152662-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.

PROTOCOLS

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