SANTA CRUZ BIOTECHNOLOGY, INC.

Rab11-FIP3 siRNA (h): sc-93428

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

1. Prekeris, R., et al. 2001. Identification of a novel Rab11/25 binding domain present in Eferin and Rip proteins. J. Biol. Chem. 276: 38966-38970.
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.

CHROMOSOMAL LOCATION

Genetic locus: RAB11FIP3 (human) mapping to 16p13.3.

PRODUCT

Rab11-FIP3 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 Rab11-FIP3 shRNA Plasmid (h): sc-93428-SH and Rab11-FIP3 shRNA (h) Lentiviral Particles: sc-93428-V as alternate gene silencing products.

For independent verification of Rab11-FIP3 (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-93428A, sc-93428B and sc-93428C.

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

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

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-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 Rab11-FIP3 gene expression knockdown using RT-PCR Primer: Rab11-FIP3 (h)-PR: sc-93428-PR (20 µl). 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.