

rabphilin-3A siRNA (m): sc-152671

BACKGROUND

The carboxy-terminal of rabphilin-3A consists of two C2 domains, A and B, and the amino-terminal (residues 45-170) contains a cysteine-rich region with two zinc finger motifs. Rabphilin-3A belongs to a family of other carboxy-terminal type (C-type) tandem C2 proteins, which includes synaptotagmins and Doc2. Rabphilin is expressed in neuroendocrine cells and co-localizes with Rab3a on synaptic vesicles and chromaffin granules. Rabphilin-3A binds Rab3a/GTP/Mg²⁺ within amino-terminal residues 45 and 170. Rabphilin-3A binds calcium ions and phosphatidylinositol 4,5-bisphosphate containing lipid vesicles within its C2 domains. Rabphilin-3A is a positive regulator of calcium dependent exocytosis, while Rab3a is a negative regulator of exocytosis. Although rabphilin-3A associates with Rab3a, they seem to influence exocytosis independently of each other. Rabphilin-3A effects are likely mediated through interactions with an unknown factor that recognizes the Rab3 binding domain.

REFERENCES

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2. Chung S.H., Joberty, G., Gelino, E.A., Macara, I.G. and Holz, R.W. 1999. Comparison of the effects on secretion in chromaffin and PC12 cells of Rab3 family members and mutants. Evidence that inhibitory effects are independent of direct interaction with rabphilin-3. *J. Biol. Chem.* 274: 18113-18120.
3. Ubach, J., Garcia, J., Nittler, M.P., Sudhof, T.C. and Rizo, J. 1999. Structure of the Janus-faced C2B domain of rabphilin. *Nat. Cell Biol.* 1: 106-112.
4. Joberty, G., Stabila, P.F., Coppola, T., Macara, I.G. and Regazzi, R. 1999. High affinity Rab3 binding is dispensable for rabphilin-dependent potentiation of stimulated secretion. *J. Cell Sci.* 112: 3579-3587.
5. Fukuda, M. and Mikoshiba, K. 2001. Synaptogmin-like protein 1-3: a novel family of C-terminal-type tandem C2 proteins. *Biochem. Biophys. Res. Commun.* 281: 1226-1233.

CHROMOSOMAL LOCATION

Genetic locus: Rph3a (mouse) mapping to 5 F.

PRODUCT

rabphilin-3A 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 rabphilin-3A shRNA Plasmid (m): sc-152671-SH and rabphilin-3A shRNA (m) Lentiviral Particles: sc-152671-V as alternate gene silencing products.

For independent verification of rabphilin-3A (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-152671A, sc-152671B and sc-152671C.

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

rabphilin-3A siRNA (m) is recommended for the inhibition of rabphilin-3A 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

rabphilin-3A (D-6): sc-393197 is recommended as a control antibody for monitoring of rabphilin-3A 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 rabphilin-3A gene expression knockdown using RT-PCR Primer: rabphilin-3A (m)-PR: sc-152671-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.