

PIPK II α siRNA (h): sc-39139

BACKGROUND

Phosphatidylinositol phosphate kinases (PIPKs) synthesize phosphatidylinositol-4,5-bisphosphate, which regulates various processes including cell proliferation, survival, membrane trafficking and cytoskeletal organization. The PIPK family is divided into three different classes, designated type I, type II and type III, each of which contain an activation loop, which determines their enzymatic specificity and subcellular targeting. The type I PIPKs (PIPK I) consist of PIPK I α , β and γ , while the type II PIPKs (PIPK II) consist of PIPK II α and β , both of which exhibit high levels of expression in the brain. Type III PIPK (designated PIP5K III) localizes to the endosome membrane where it participates in endosome-related membrane trafficking and, like other PIPK proteins, generates phosphatidylinositol-4,5-bisphosphate via ATP-dependent phosphorylation. Due to their ability to regulate phosphatidylinositol-4,5-bisphosphate production, the PIPK proteins are essential messengers for signal transduction pathways throughout the body.

REFERENCES

1. Carricaburu, V., et al. 2003. The phosphatidylinositol (PI)-5-phosphate 4-kinase type II enzyme controls Insulin signaling by regulating PI-3,4,5-trisphosphate degradation. *Proc. Natl. Acad. Sci. USA* 100: 9867-9872.
2. Cabezas, A., et al. 2006. Cloning and subcellular localization of a human phosphatidylinositol 3-phosphate 5-kinase, PIKfyve/Fab1. *Gene* 371: 34-41.
3. Rutherford, A.C., et al. 2006. The mammalian phosphatidylinositol 3-phosphate 5-kinase (PIKfyve) regulates endosome-to-TGN retrograde transport. *J. Cell Sci.* 119: 3944-3957.
4. Clarke, J.H., et al. 2007. Type II PtdInsP kinases: location, regulation and function. *Biochem. Soc. Symp.* 74: 149-159.
5. Karataeva, N.A. and Nevinsky, G.A. 2007. Enzymes phosphorylating lipids and polysaccharides. *Biochemistry* 72: 367-379.
6. Kanaho, Y., et al. 2007. The phosphoinositide kinase PIP5K that produces the versatile signaling phospholipid PI4,5P₂. *Biol. Pharm. Bull.* 30: 1605-1609.
7. Sinha, R.K. and Subrahmanyam, G. 2007. Type II phosphatidylinositol 4-kinase(s) in cell signaling cascades. *Indian J. Biochem. Biophys.* 44: 289-294.

CHROMOSOMAL LOCATION

Genetic locus: PIP5K2A (human) mapping to 10p12.2.

PRODUCT

PIPK II α 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see PIPK II α shRNA Plasmid (h): sc-39139-SH and PIPK II α shRNA (h) Lentiviral Particles: sc-39139-V as alternate gene silencing products.

For independent verification of PIPK II α (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-39139A, sc-39139B and sc-39139C.

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

PIPK II α siRNA (h) is recommended for the inhibition of PIPK II α 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 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

PIPK II (D-3): sc-393246 is recommended as a control antibody for monitoring of PIPK II α 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 PIPK II α gene expression knockdown using RT-PCR Primer: PIPK II α (h)-PR: sc-39139-PR (20 μ l, 428 bp). 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.