SANTA CRUZ BIOTECHNOLOGY, INC.

PIP5KIII siRNA (m): sc-72205

BACKGROUND

Phosphorylation of phosphatidylinositol (PtdIns) derivatives is suggested to play a role in regulating cytoskeletal functions, membrane trafficking and receptor signaling by recruiting protein complexes to cell- and endosomal-membranes. PtdIns proteins are distinguished by the degree and position of phosphorylation of the inositol ring. PIP5KIII (Phosphatidylinositol 3-phosphate 5-kinase), also known as PIP5K3, FAB1, CFD, PIKFYVE or ZFYVE29 is 2,098 amino acid protein that synthesizes phosphatidylinositol-3,5-bisphosphate by catalyzing the phosphorylation of phosphatidylinositol 3-phosphate on the fifth hydroxyl of the inositol ring. PIP5KIII is thought to play a major role in nuclear migration and the endocytic-vacuolar pathway. PIP5KIII exists as four alternatively spliced isoforms and contains a DEP domain, a FYVE-type zinc finger and a PIPK domain. Defects in the gene encoding PIP5KIII lead to corneal fleck dystrophy (CFD), an autosomal disorder in which small white flecks are found throughout the corneal stroma.

REFERENCES

1. Sbrissa, D., et al. 2002. Phosphatidylinositol 3-phosphate-interacting domains in PIKfyve. Binding specificity and role in PIKfyve. Endomenbrane localization. J. Biol. Chem. 277: 6073-6079.
2. Ikonomov, O.C., et al. 2003. PIKfyve controls fluid phase endocytosis but not recycling/degradation of endocytosed receptors or sorting of procathepsin D by regulating multivesicular body morphogenesis. Mol. Biol. Cell 14: 4581-4591.
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. Kim, J., et al. 2007. The phosphoinositide kinase PIKfyve mediates epidermal growth factor receptor trafficking to the nucleus. Cancer Res. 67: 9229-9237.
5. Shisheva, A., et al. 2008. PIKfyve: Partners, significance, debates and paradoxes. Cell Biol. Int. 32: 591-604.

CHROMOSOMAL LOCATION

Genetic locus: Pip5k3 (mouse) mapping to 1 C2.

PRODUCT

PIP5KIII 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see PIP5KIII shRNA Plasmid (m): sc-72205-SH and PIP5KIII shRNA (m) Lentiviral Particles: sc-72205-V as alternate gene silencing products.

For independent verification of PIP5KIII (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-72205A, sc-72205B and sc-72205C.

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 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

PIP5KIII siRNA (m) is recommended for the inhibition of PIP5KIII 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 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

PIP5KIII (64-Q6): sc-100408 is recommended as a control antibody for monitoring of PIP5KIII 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 PIP5KIII gene expression knockdown using RT-PCR Primer: PIP5KIII (m)-PR: sc-72205-PR (20 µl, 419 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.