SANTA CRUZ BIOTECHNOLOGY, INC.

PI 4-kinase β siRNA (h): sc-45716

BACKGROUND

The members of the phosphatidylinositol kinase (PIK) superfamily can be divided into three groups based on their substrate specificity. PIKs convert phosphatidylinositol (PI) into PI phosphate [PI(3)P], PI phosphate [PI(4)P], PI bisphosphate [PI(4, 5)P2] and PI triphosphate [PI(3, 4,5)P3]. The first group, the PI 3-kinases, is composed of highly related proteins designated p110α, p110β, p110γ and p110δ which convert PI into PI(3)P and PI(4, 5)P2 into PI(3, 4, 5)P3. The second group, the PI 4-kinases, convert PI into PI(4)P. The third group, the PI(4)P5-kinases, convert PI(4)P into PI(4, 5)P2. Phosphatidylinositides represent important regulatory molecules and are involved in a diverse array of signaling pathways. Phosphatidylinositol biphosphate acts as an activator of PKCs and as a substrate for PLC γ, which converts the molecule into the second messengers, inositol-1, 4, 5 triphosphate and 1, 2-diacylglycerol. PI(3, 4, 5)P3 has been shown to activate the PKC ζ isoform. PI 4-kinase β is a cytoplasmic protein inhibited by wortmannin.

REFERENCES

1. Woscholski, R., et al. 1994. Biochemical characterization of the free catalytic p110α and the complexed heterodimeric p110α.p85α forms of the mammalian phosphatidylinositol 3-kinase. J. Biol. Chem. 269: 25067-25072.
2. Woscholski, R., et al. 1994. A comparison of demethoxyviridin and wortmannin as inhibitors of phosphatidylinositol 3-kinase. FEBS Lett. 342: 109-114.
3. Hunter, T., et al. 1995. When is a lipid kinase not a lipid kinase? When it is a protein kinase. Cell 83: 1-4.
4. Zhou, K., et al. 1995. A phosphatidylinositol (PI) kinase gene family in *Dictyostelium discoideum:* biological roles of putative mammalian p110 and yeast Vps34p PI 3-kinase homologs during growth and development. Mol. Cell. Biol. 15: 5645-5656.
5. Wong, K., et al. 1997. Subcellular locations of phosphatidylinositol 4-kinase isoforms. J. Biol. Chem. 272: 13236-13241.
6. Godi, A., et al. 1999. ARF mediates recruitment of PtdIns-4-OH kinase-β and stimulates synthesis of PtdIns(4,5)P2 on the Golgi complex. Nat. Cell Biol. 1: 280-287.

CHROMOSOMAL LOCATION

Genetic locus: PI4KB (human) mapping to 1q21.3.

PRODUCT

PI 4-kinase β 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 PI 4-kinase β shRNA Plasmid (h): sc-45716-SH and PI 4-kinase β shRNA (h) Lentiviral Particles: sc-45716-V as alternate gene silencing products.

For independent verification of PI 4-kinase β (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-45716A, sc-45716B and sc-45716C.

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

PI 4-kinase β siRNA (h) is recommended for the inhibition of PI 4-kinase β 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

PI 4-kinase β (E-4): sc-166615 is recommended as a control antibody for monitoring of PI 4-kinase β 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 PI 4-kinase β gene expression knockdown using RT-PCR Primer: PI 4-kinase β (h)-PR: sc-45716-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.