SANTA CRUZ BIOTECHNOLOGY, INC.

PITPβ siRNA (h): sc-76150

BACKGROUND

The lipid binding proteins known as phosphatidylinositol transfer proteins (PITP) facilitate the formation of phosphatidylinositol derived second messenger molecules, which are related to the phospholipase C and phosphoinositide 3-kinase pathways. PITP are ubiquitously expressed proteins that transfer phosphatidylinositol (PI) and phosphatidylcholine (PC) between membranes enriched in PI or PC to membranes that are deficient in PI or PC. PITP mobilizes PI from the endoplasmic recticulum and regulates the release of PI from stored vesicles in the Golgi network. In mammalian cells, three smaller forms of soluble PITP are present, designated PITPα, PITPβ and retinal degeneration B (rdgB) β. PITPβ is a 271 amino acid protein that is widely expressed in various tissues. Though required for Golgi targeting, constitutive phosphorylation of Ser 262 has no effect on phospholipid transfer activity. There are two isoforms of PITPβ that are produced as a result of alternative splicing events.

REFERENCES

1. Tanaka, S., et al. 1995. Cloning and expression of human cDNA encoding phosphatidylinositol transfer protein β. Biochim. Biophys. Acta 1259: 199-202.
2. Cockcroft, S. 1999. Mammalian phosphatidylinositol transfer proteins: emerging roles in signal transduction and vesicular traffic. Chem. Phys. Lipids 98: 23-33.
3. Segui, B., et al. 2002. Phosphatidylinositol transfer protein β displays minimal sphingomyelin transfer activity and is not required for biosynthesis and trafficking of sphingomyelin. Biochem. J. 366: 23-34.
4. Online Mendelian Inheritance in Man, OMIM™. 2002. Johns Hopkins University, Baltimore, MD. MIM Number: 606876. World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/
5. Vordtriede, P.B., et al. 2005. Structure of PITPβ in complex with phosphatidylcholine: comparison of structure and lipid transfer to other PITP isoforms. Biochemistry 44: 14760-14771.
6. Phillips, S.E., et al. 2006. Specific and nonspecific membrane-binding determinants cooperate in targeting phosphatidylinositol transfer protein β-isoform to the mammalian *trans*-Golgi network. Mol. Biol. Cell 17: 2498-2512.

CHROMOSOMAL LOCATION

Genetic locus: PITPNB (human) mapping to 22q12.1.

PRODUCT

PITPβ 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 PITPβ shRNA Plasmid (h): sc-76150-SH and PITPβ shRNA (h) Lentiviral Particles: sc-76150-V as alternate gene silencing products.

For independent verification of PITPβ (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-76150A, sc-76150B and sc-76150C.

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

PITPβ siRNA (h) is recommended for the inhibition of PITPβ 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

PITPβ (G-3): sc-390500 is recommended as a control antibody for monitoring of PITPβ 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 PITPβ gene expression knockdown using RT-PCR Primer: PITPβ (h)-PR: sc-76150-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.