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SANTA CRUZ BIOTECHNOLOGY, INC.

PC-PLD2 siRNA (h): sc-44001

BACKGROUND

Virtually every cell uses phosphatidylcholine as a substrate to produce phosphatidic acid and choline. Phosphatidylcholine phospholipase D1 and D2 (PC-PLD1 and PC-PLD2) are phospholipid-specific phosphodiesterases that hydrolyze phosphatidylcholine. Unlike PC-PLD1, which associates with secretory granules, PC-PLD2 localizes to the plasma membrane, where it is implicated in the formation of endocytotic vesicles. Both PC-PLD1 and PC-PLD2 coordinately regulate macrophage phagocytosis. PC-PLD activity in mammalian cells is transiently stimulated upon activation by G protein-coupled and receptor tyrosine kinase cell surface receptors. For example, PC-PLD1 and PC-PLD2 participate in sphingosine 1-phosphate stimulation of ERK phosphorylation and IL-8 secretion in bronchial epithelial cells. In addition, tubulin binding to PC-PLD2 inhibits muscarinic receptor-linked PC-PLD2 activation. PC-PLD2 also enhances PKCζ activity through direct interaction in a lipase activity-independent manner. PC-PLD1 and PC-PLD2 stimulate cell growth by repressing expression of p21 gene through p53-dependent and p53-independent pathways, respectively, which may ultimately lead to carcinogenesis.

REFERENCES

1. Nishida, A., et al. 1994. Brain ischemia decreases phosphatidylcholine-phospholipase D but not phosphatidylinositol phospholipase C in rats. Stroke 25: 1247-1251.
2. del Peso, L., et al. 1996. Activation of phospholipase D by Ras proteins is independent of protein kinase C. J. Cell. Biochem. 61: 599-608.
3. Houle, M.G., et al. 1999. Regulation of phospholipase D by phosphorylation-dependent mechanisms. Biochim. Biophys. Acta 1439: 135-149.
4. Cockcroft, S. 2001. Signalling roles of mammalian phospholipase D1 and D2. Cell. Mol. Life Sci. 58: 1674-1687.
5. Zhao, D., et al. 2001. Generation of choline for acetylcholine synthesis by phospholipase D isoforms. BMC Neurosci. 2: 16.

CHROMOSOMAL LOCATION

Genetic locus: PLD2 (human) mapping to 17p13.2.

PRODUCT

PC-PLD2 siRNA (h) is a target-specific 19-25 nt siRNA 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 PC-PLD2 shRNA Plasmid (h): sc-44001-SH and PC-PLD2 shRNA (h) Lentiviral Particles: sc-44001-V as alternate gene silencing 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

PC-PLD2 siRNA (h) is recommended for the inhibition of PC-PLD2 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

PC-PLD2 (B-3): sc-515744 is recommended as a control antibody for monitoring of PC-PLD2 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 PC-PLD2 gene expression knockdown using RT-PCR Primer: PC-PLD2 (h)-PR: sc-44001-PR (20 µl, 497 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Usatyuk, P.V., et al. 2009. Phospholipase D-mediated activation of IQGAP1 through Rac 1 regulates hyperoxia-induced p47phox translocation and reactive oxygen species generation in lung endothelial cells. J. Biol. Chem. 284: 15339-15352.
2. Chen, F., et al. 2013. Phospholipase D2 mediates signaling by ATPase class I type 8B membrane 1. J. Lipid Res. 54: 379-385.

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.