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- Trockeneiszuschlag
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SANTA CRUZ BIOTECHNOLOGY, INC.

PC-PLD1 shRNA (h) Lentiviral Particles: sc-44000-V

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: PLD1 (human) mapping to 3q26.31.

PRODUCT

PC-PLD1 shRNA (h) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 µl frozen stock containing 1.0 x 10^6 infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see PC-PLD1 siRNA (h): sc-44000 and PC-PLD1 shRNA Plasmid (h): sc-44000-SH as alternate gene silencing products.

APPLICATIONS

PC-PLD1 shRNA (h) Lentiviral Particles is recommended for the inhibition of PC-PLD1 expression in human cells.

PROTOCOLS

See our web site at www.scbt.com or our catalog for detailed protocols and support products.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 µl frozen viral stock containing 1.0 x 10^6 infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

GENE EXPRESSION MONITORING

PC-PLD1 (F-12): sc-28314 is recommended as a control antibody for monitoring of PC-PLD1 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 (secondary) reagents are recommended: 1) Western Blotting: use goat anti-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker™ compatible goat anti-mouse IgG-HRP: sc-2031 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor PC-PLD1 gene expression knockdown using RT-PCR Primer: PC-PLD1 (h)-PR: sc-44000-PR (20 µl, 437 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

STORAGE

Store lentiviral particles at -80° C. Stable for at least one year from the date of shipment. Once thawed, particles can be stored at 4° C for up to one week. Avoid repeated freeze thaw cycles.

RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.