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PLC ϵ shRNA (h) Lentiviral Particles: sc-44024-V

BACKGROUND

Phosphoinositide-specific phospholipase C (PLC) plays a crucial role in the initiation of receptor mediated signal transduction through the generation of the two second messengers, inositol 1,4,5-triphosphate and diacylglycerol from phosphatidylinositol 4,5-bisphosphate. There are many mammalian PLC isozymes, including PLC β 1, PLC β 2, PLC β 3, PLC β 4, PLC γ 1, PLC γ 2, PLC δ 1, PLC δ 2 and PLC ϵ . Phospholipase C ϵ (PLC ϵ) is characterized by possession of CDC25 homology and Ras/Rap1-associating domains. PLC ϵ is translocated from the cytoplasm to the plasma membrane and activated by direct association with Ras at its Ras/Rap1-associating domain.

REFERENCES

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2. Kelley, G.G., et al. 2001. Phospholipase C ϵ : a novel Ras effector. *EMBO J.* 20: 743-754.
3. Jin, T.G., et al. 2001. Role of the CDC25 homology domain of phospholipase C ϵ in amplification of Rap1-dependent signaling. *J. Biol. Chem.* 276: 30301-30307.
4. Wing, M.R., et al. 2001. Activation of phospholipase C ϵ by heterotrimeric G protein $\beta\gamma$ -subunits. *J. Biol. Chem.* 276: 48257-48261.
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CHROMOSOMAL LOCATION

Genetic locus: PLCE1 (human) mapping to 10q23.33.

PRODUCT

PLC ϵ 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 μ l frozen stock containing 1.0×10^6 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 PLC ϵ siRNA (h): sc-44024 and PLC ϵ shRNA Plasmid (h): sc-44024-SH as alternate gene silencing products.

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.

APPLICATIONS

PLC ϵ shRNA (h) Lentiviral Particles is recommended for the inhibition of PLC ϵ expression in human cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0×10^6 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

PLC ϵ (N-20): sc-28402 is recommended as a control antibody for monitoring of PLC ϵ 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 donkey anti-goat IgG-HRP: sc-2020 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible donkey anti-goat IgG-HRP: sc-2033 (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 donkey anti-goat IgG-FITC: sc-2024 (dilution range: 1:100-1:400) or donkey anti-goat IgG-TR: sc-2783 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor PLC ϵ gene expression knockdown using RT-PCR Primer: PLC ϵ (h)-PR: sc-44024-PR (20 μ l, 427 bp). Annealing temperature for the primers should be $55-60^\circ$ C and the extension temperature should be $68-72^\circ$ 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.

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.

SELECT PRODUCT CITATIONS

1. Baljinnyam, E., et al. 2010. Exchange protein directly activated by cyclic AMP increases melanoma cell migration by a Ca^{2+} -dependent mechanism. *Cancer Res.* 70: 5607-5617.