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# PKC $\epsilon$ shRNA (h2) Lentiviral Particles: sc-44228-V

## BACKGROUND

Members of the protein kinase C (PKC) family play a key regulatory role in a variety of cellular functions including cell growth and differentiation, gene expression, hormone secretion and membrane function. PKCs were originally identified as serine/threonine protein kinases whose activity was dependent on calcium and phospholipids. Diacylglycerols (DAG) and tumor promoting phorbol esters bind to and activate PKC. PKCs can be subdivided into many different isoforms ( $\alpha$ ,  $\beta$ I,  $\beta$ II,  $\gamma$ ,  $\delta$ ,  $\epsilon$ ,  $\zeta$ , h,  $\lambda$ , n,  $\mu$ ,  $\nu$ , and  $\theta$ ). Patterns of expression for each PKC isoform differ among tissues and PKC family members exhibit clear differences in their cofactor dependencies. For instance, the kinase activities of PKC  $\delta$  and  $\epsilon$  are independent of  $Ca^{++}$ . On the other hand, most of the other PKC members possess phorbol ester-binding activities and kinase activities.

## REFERENCES

1. Takai, Y., et al. 1979. Calcium-dependent activation of a multifunctional protein kinase by membrane phospholipids. *J. Biol. Chem.* 254: 3692-3695.
2. Castagna, M., et al. 1982. Direct activation of calcium-activated, phospholipid-dependent protein kinase by tumor-promoting phorbol esters. *J. Biol. Chem.* 257: 7847-7851.
3. Kikkawa, U., et al. 1983. Protein kinase C as a possible receptor of tumor-promoting phorbol esters. *J. Biol. Chem.* 258: 11442-11445.
4. Nishizuka, Y. 1984. The role of protein kinase C in cell surface signal transduction and tumour promotion. *Nature* 308: 693-698.
5. Nishizuka, Y. 1984. Turnover of inositol phospholipids and signal transduction. *Science* 225: 1365-1370.
6. Ohno, S., et al. 1991. Structural and functional diversities of a family of signal transducing protein kinases, protein kinase C family; two distinct classes of PKC, conventional cPKC and novel nPKC. *Adv. Enzyme Regul.* 31: 287-303.
7. Olivier, A.R., et al. 1991. Expression and characterization of protein kinase C-delta. *Eur. J. Biochem.* 200: 805-810.

## CHROMOSOMAL LOCATION

Genetic locus: PRKCE (human) mapping to 2p21.

## PRODUCT

PKC  $\epsilon$  shRNA (h2) 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  $\mu$ l frozen stock containing  $1.0 \times 10^6$  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 PKC  $\epsilon$  siRNA (h2): sc-44228 and PKC  $\epsilon$  shRNA Plasmid (h2): sc-44228-SH as alternate gene silencing products.

## STORAGE

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

## APPLICATIONS

PKC  $\epsilon$  shRNA (h2) Lentiviral Particles is recommended for the inhibition of PKC  $\epsilon$  expression in human cells.

## SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200  $\mu$ l frozen viral stock containing  $1.0 \times 10^6$  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

PKC  $\epsilon$  C-15): sc-214 is recommended as a control antibody for monitoring of PKC  $\epsilon$  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-rabbit IgG-HRP: sc-2004 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible goat anti-rabbit IgG-HRP: sc-2030 (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-rabbit IgG-FITC: sc-2012 (dilution range: 1:100-1:400) or goat anti-rabbit IgG-TR: sc-2780 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

## RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor PKC  $\epsilon$  gene expression knockdown using RT-PCR Primer: PKC  $\epsilon$  (h2)-PR: sc-44228-PR (20  $\mu$ l, 515 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.

## PROTOCOLS

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