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PKC ϵ siRNA (h2): sc-44228

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 at least two major classes, including conventional (c) PKC isoforms (α , β I, β II and γ) and novel (n) PKC isoforms (δ , ϵ , ζ , η , θ , λ /I, ν and ν). 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 δ and ϵ are independent of Ca^{2+} . 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.

CHROMOSOMAL LOCATION

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

PRODUCT

PKC ϵ siRNA (h2) 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see PKC ϵ shRNA Plasmid (h2): sc-44228-SH and PKC ϵ shRNA (h2) Lentiviral Particles: sc-44228-V as alternate gene silencing products.

For independent verification of PKC ϵ (h2) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-44228A, sc-44228B and sc-44228C.

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 μ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330 μ l of RNase-free water makes a 10 μ M solution in a 10 μ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

PKC ϵ siRNA (h2) is recommended for the inhibition of PKC ϵ 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 μ M in 66 μ l. 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

PKC ϵ (E-5): sc-1681 is recommended as a control antibody for monitoring of PKC 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 reagents are recommended: 1) Western Blotting: use m-IgG κ BP-HRP: sc-516102 or m-IgG κ 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-IgG κ BP-FITC: sc-516140 or m-IgG κ 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 PKC ϵ gene expression knockdown using RT-PCR Primer: PKC ϵ (h2)-PR: sc-44228-PR (20 μ l, 515 bp). Annealing temperature for the primers should be 55-60 $^{\circ}$ C and the extension temperature should be 68-72 $^{\circ}$ C.

SELECT PRODUCT CITATIONS

1. Kedei, N., et al. 2011. The synthetic bryostatin analog Merle 23 dissects distinct mechanisms of bryostatin activity in the LNCaP human prostate cancer cell line. *Biochem. Pharmacol.* 81: 1296-1308.

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.