

PKC ϵ siRNA (m): sc-36250

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, μ and ν). Patterns of expression for each PKC isoform differs 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.

CHROMOSOMAL LOCATION

Genetic locus: Prkce (mouse) mapping to 17 E4.

PRODUCT

PKC ϵ siRNA (m) 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 μM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see PKC ϵ shRNA Plasmid (m): sc-36250-SH and PKC ϵ shRNA (m) Lentiviral Particles: sc-36250-V as alternate gene silencing products.

For independent verification of PKC ϵ (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-36250A, sc-36250B and sc-36250C.

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

PKC ϵ siRNA (m) is recommended for the inhibition of PKC ϵ expression in mouse 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

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 MarkerTM 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 (m)-PR: sc-36250-PR (20 μl , 547 bp). Annealing temperature for the primers should be $55-60^{\circ}\text{C}$ and the extension temperature should be $68-72^{\circ}\text{C}$.

SELECT PRODUCT CITATIONS

- Wen, J., et al. 2011. Specific PKC isoforms regulate LPS-stimulated iNOS induction in murine microglial cells. *J. Neuroinflammation* 8: 38.
- Merighi, S., et al. 2013. Morphine mediates a proinflammatory phenotype via μ -opioid receptor-PKC ϵ -Akt-ERK1/2 signaling pathway in activated microglial cells. *Biochem. Pharmacol.* 86: 487-496.
- Nishizaki, T., et al. 2016. The phosphatidylethanolamine derivative diDCP-LA-PE mimics intracellular Insulin signaling. *Sci. Rep.* 6: 27267.
- Gessi, S., et al. 2016. The activation of μ -opioid receptor potentiates LPS-induced NF κ B promoting an inflammatory phenotype in microglia. *FEBS Lett.* 590: 2813-2826.
- Chiu, Y.T., et al. 2017. Agonist-dependent and -independent κ opioid receptor phosphorylation: distinct phosphorylation patterns and different cellular outcomes. *Mol. Pharmacol.* 92: 588-600.
- Nishizaki, T. 2018. Dioleoylphosphoethanolamine retains cell surface GLUT4 by inhibiting PKC α -driven internalization. *Cell. Physiol. Biochem.* 46: 1985-1998.
- Di Pietro, P., et al. 2022. Targeting the ASMAse/S1P pathway protects from sortilin-evoked vascular damage in hypertension. *J. Clin. Invest.* 132: e146343.

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.