

# SignalSilence® PKC $\zeta$ siRNA II

✓ 10  $\mu$ M in 300  $\mu$ l  
(3 nmol)



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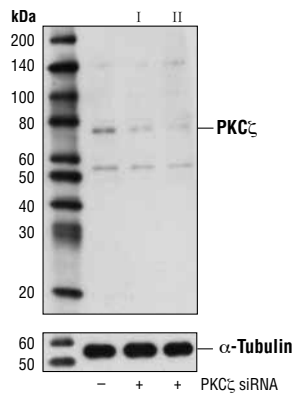
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**For Research Use Only. Not For Use In Diagnostic Procedures.**

## Species Cross-Reactivity: H

**Description:** SignalSilence® PKC $\zeta$  siRNA II from Cell Signaling Technology (CST) allows the researcher to specifically inhibit PKC $\zeta$  expression by RNA interference, a method whereby gene expression can be selectively silenced through the delivery of double stranded RNA molecules into the cell. All SignalSilence® siRNA products from CST are rigorously tested in-house and have been shown to reduce target protein expression by western analysis.

**Background:** Activation of protein kinase C (PKC) is one of the earliest events in a cascade that controls a variety of cellular responses, including secretion, gene expression, proliferation, and muscle contraction (1,2). PKC isoforms belong to three groups based on calcium dependency and activators. Classical PKCs are calcium-dependent via their C2 domains and are activated by phosphatidylserine (PS), diacylglycerol (DAG), and phorbol esters (TPA, PMA) through their cysteine-rich C1 domains. Both novel and atypical PKCs are calcium-independent, but only novel PKCs are activated by PS, DAG, and phorbol esters (3-5). Members of these three PKC groups contain a pseudo-substrate or autoinhibitory domain that binds to substrate-binding sites in the catalytic domain to prevent activation in the absence of cofactors or activators. Control of PKC activity is regulated through three distinct phosphorylation events. Phosphorylation at Thr500 in the activation loop, the autophosphorylation site at Thr641, and at carboxy-terminal hydrophobic site Ser660 occurs *in vivo* (2). Atypical PKC isoforms lack hydrophobic region phosphorylation, which correlates with the presence of glutamic acid rather than the serine or threonine residues found in more typical PKC isoforms. Either the enzyme PDK1 or a close relative is responsible for PKC activation. A recent addition to the PKC superfamily is PKC $\mu$  (PKD), which is regulated by DAG and TPA through its C1 domain. PKD is distinguished by the presence of a PH domain and by its unique substrate recognition and Golgi localization (6). PKC-related kinases (PRK) lack the C1 domain and do not respond to DAG or phorbol esters. Phosphatidylinositol lipids activate PRKs and small Rho-family GTPases bind to the homology region 1 (HR1) to regulate PRK kinase activity (7).



Western blot analysis of extracts from HeLa cells, transfected with 100 nM SignalSilence® Control siRNA (Unconjugated) #6568 (-), SignalSilence® PKC $\zeta$  siRNA I #8672 (+), or SignalSilence® PKC $\zeta$  siRNA II (+), using PKC $\zeta$  (C24E6) Rabbit mAb #9368 (upper) or  $\alpha$ -Tubulin (11H10) Rabbit mAb #2125 (lower). The PKC $\zeta$  (C24E6) Rabbit mAb confirms silencing of PKC $\zeta$  expression, while the  $\alpha$ -Tubulin (11H10) Rabbit mAb is used as a loading control

**Directions for Use:** CST recommends transfection with 100 nM SignalSilence® PKC $\zeta$  siRNA II 48 to 72 prior to cell lysis. For transfection procedure, follow protocol provided by the transfection reagent manufacturer. Please feel free to contact CST with any questions on use.

Each vial contains the equivalent of 100 transfections, which corresponds to a final siRNA concentration of 100 nM per transfection in a 24-well plate with a total volume of 300  $\mu$ l per well.

**Quality Control:** Oligonucleotide synthesis is monitored base by base through trityl analysis to ensure appropriate coupling efficiency. The oligo is subsequently purified by affinity-solid phase extraction. The annealed RNA duplex is further analyzed by mass spectrometry to verify the exact composition of the duplex. Each lot is compared to the previous lot by mass spectrometry to ensure maximum lot-to-lot consistency.

Entrez-Gene ID #5590  
Swiss-Prot Acc. #Q05513

**Storage:** PKC $\zeta$  siRNA II is supplied in RNase-free water. Aliquot and store at -20 °C.

**Please visit [www.cellsignal.com](http://www.cellsignal.com) for a complete listing of recommended companion products.**

## Background References:

- (1) Nishizuka, Y. (1984) *Nature* 308, 693-698.
- (2) Keranen, L.M. et al. (1995) *Curr. Biol.* 5, 1394-1403.
- (3) Mellor, H. and Parker, P.J. (1998) *Biochem J.* 332 (Pt 2), 281-292.
- (4) Ron, D. and Kazanietz, M.G. (1999) *FASEB J.* 13, 1658-1676.
- (5) Moscat, J. and Diaz-Meco, M.T. (2000) *EMBO Rep.* 1, 399-403.
- (6) Baron, C.L. and Malhotra, V. (2002) *Science* 295, 325-328.
- (7) Flynn, P. et al. (2000) *J. Biol. Chem.* 275, 11064-11070.