SignalSilence® PKA C-α siRNA II

#6574 Store at -20°C

☑ 10 µM in 300 µl
(100 Transfections)

Cell Signaling TECHNOLOGY®

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

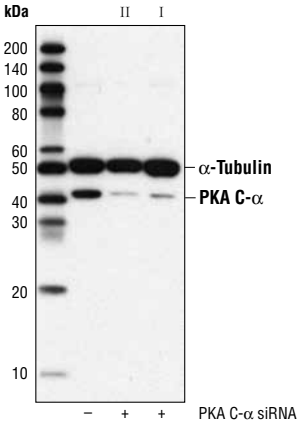
Species Cross-Reactivity: H, (M, R)

Description: SignalSilence® PKA C-α siRNA II from Cell Signaling Technology (CST) allows the researcher to specifically inhibit PKA C-α expression using 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 are rigorously tested in-house and have been shown to reduce target protein expression by western analysis.

Background: The second messenger cyclic AMP (cAMP) activates cAMP-dependent protein kinase (PKA or cAPK) in mammalian cells and controls many cellular mechanisms such as gene transcription, ion transport and protein phosphorylation (1). Inactive PKA is a heterotetramer composed of a regulatory subunit (R) dimer and a catalytic subunit (C) dimer. In this inactive state, the pseudosubstrate sequences on the R subunits block the active sites on the C subunits. Three C subunit isoforms (C-α, C-β and C-γ) and two families of regulatory subunits (RI and RII) with distinct cAMP binding properties have been identified. Within the two R families, two isoforms, α and β (RI-α, RI-β, RII-α and RII-β) exist. Upon binding of cAMP to the R subunits, the autoinhibitory contact is eased and active monomeric C subunits are released. PKA shares substrate specificity with Akt (PKB) and PKC, which is characterized by an arginine at position -3 relative to the phosphorylated serine or threonine residue (2). Substrates that present this consensus sequence and have been shown to be phosphorylated by PKA are Bad (Ser155), CREB (Ser133) and GSK-3 (GSK-3α Ser21 and GSK-3β Ser9) (3-5). In addition, combined knock-down of PKA C-α and -β blocks cAMP-mediated phosphorylation of Raf (Ser43 and Ser259) (6). Autophosphorylation and phosphorylation by PDK-1 are two known mechanisms responsible for phosphorylation of the C subunit at Thr197 (7).

Directions for Use: CST recommends transfection with 100 nM PKA C-α siRNA 48 to 72 hours 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.

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.



Western blot analysis of extracts from HeLa cells, transfected with either 100 nM SignalSilence® Control siRNA (Fluorescein Conjugate) #6201 (-), SignalSilence® PKA C-α siRNA II (+) or SignalSilence® PKA C-α siRNA I #6406 (+), using PKA C-α Antibody #4782 and α-Tubulin (11H10) Rabbit mAb #2125. The PKA C-α antibody confirms silencing of PKA C-α expression and α-Tubulin (11H10) Rabbit mAb is used to control for loading and specificity of PKA C-α siRNA.

Specificity/ Sensitivity: SignalSilence® PKA C-α siRNA II will inhibit human, mouse and rat PKA C-α exoression.

Entrez-Gene ID #5566
Swiss-Prot Acc. #P17612

Storage: PKA C-α siRNA II is supplied in RNAse-free water. Aliquot and store at -20ºC.

Please visit www.cellsignal.com for a complete listing of recommended companion products.

Background References:

(1) Montminy, M. (1997) *Annu. Rev. Biochem.* 66, 807–822.

(2) Dell'Acqua, M.L. and Scott, J.D. (1997) *J. Biol. Chem.* 272, 12881–12884.

(3) Tan, Y. et al. (2000) *J. Biol. Chem.* 275, 25865–25869.

(4) Gonzalez, G.A. and Montminy, M.R. (1989) *Cell* 59, 675–680.

(5) Fang, X. et al. (2000) *Proc. Natl. Acad. Sci. USA* 97, 11960–11965.

(6) Dumaz, N. and Marais, R. (2003) *J. Biol. Chem.* 278, 29819 –29823.

(7) Moore, M.J. et al. (2002) *J. Biol. Chem.* 277, 47878–47884.

Applications Key: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide

Species Cross-Reactivity Key: **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **Ce**—C. elegans **Hr**—Horse **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.