

SignalSilence® Akt siRNA I

10 µM in 300 µl
(100 Transfections)



Cell Signaling

TECHNOLOGY®

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This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals.

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

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

Isoform Activity: SignalSilence® Akt siRNA I inhibits expression of Akt1 and Akt2, but does not inhibit Akt3 expression.

Background: Akt, also referred to as PKB or Rac, plays a critical role in controlling survival and apoptosis (1-3). This protein kinase is activated by insulin and various growth and survival factors to function in a wortmannin-sensitive pathway involving PI3 kinase (2,3). Akt is activated by phospholipid binding and activation loop phosphorylation at Thr308 by PDK1 (4) and by phosphorylation within the carboxy terminus at Ser473. The previously elusive PDK2 responsible for phosphorylation of Akt at Ser473 has been identified as mammalian target of rapamycin (mTor) in a rapamycin-insensitive complex with rictor and Sin1 (5,6). Akt promotes cell survival by inhibiting apoptosis by phosphorylating and inactivating several targets, including Bad (7), forkhead transcription factors (8), c-Raf (9) and caspase-9. PTEN phosphatase is a major negative regulator of the PI3 kinase/Akt signaling pathway (10). LY294002 is a specific PI3 kinase inhibitor (11).

Another essential Akt function is the regulation of glycogen synthesis through phosphorylation and inactivation of GSK-3 α and β (12,13). Akt may also play a role in insulin stimulation of glucose transport (12).

In addition to its role in survival and glycogen synthesis, Akt is involved in cell cycle regulation by preventing GSK-3 β mediated phosphorylation and degradation of cyclin D1 (14) and by negatively regulating the cyclin dependent kinase inhibitors p27 Kip (15) and p21 Waf1/CIP1 (16). Akt also plays a critical role in cell growth by directly phosphorylating mTOR in a rapamycin-sensitive complex containing raptor (17). More importantly, Akt phosphorylates and inactivates tuberin (TSC2), an inhibitor of mTOR within the mTOR-raptor complex (18). Inhibition of mTOR stops the protein synthesis machinery by inactivating p70 S6 kinase and activating the eukaryotic initiation factor 4E binding protein 1 (4E-BP1), an inhibitor of translation (18,19).

Small interfering RNA (siRNA) has been used to specifically silence Akt in CHO cells and 3T3-L1 adipocytes (20).

Specificity/Sensitivity: SignalSilence® Akt siRNA I will inhibit human, mouse and rat Akt1 and Akt2 expression. It will not affect Akt3 expression.

Directions for Use: CST recommends transfection with 100 nM Akt siRNA I 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.

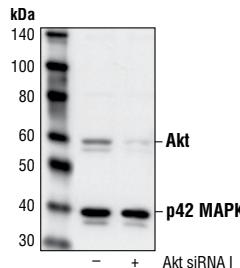
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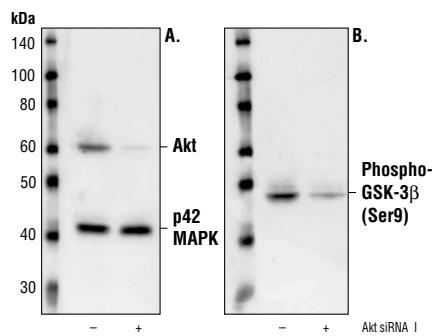
Entrez-Gene ID #207
Swiss-Prot Acc. #P31749

Storage: Akt siRNA I is supplied in RNase-free water. Aliquot and store at -20°C.

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



Western blot analysis of extracts from HeLa cells, transfected with 100 nM SignalSilence® Control siRNA (Fluorescein Conjugate) #6201 (-) or SignalSilence® Akt siRNA I (+), using Akt Antibody #9272 and p42 MAP Kinase (Erk2) Antibody #9108. Akt antibody confirms silencing of Akt expression, while the p42 MAP kinase (Erk2) antibody is used to control for loading and specificity of Akt siRNA.



Western blot analysis of extracts from CHO cells, transfected with non-targeted (-) or SignalSilence® Akt siRNA I (+) siRNA, using Akt Antibody #9272 and p42 MAP Kinase (Erk2) Antibody #9108. The Akt antibody confirms silencing of protein expression while the p42 MAP Kinase (Erk2) antibody was used to control for loading and specificity of Akt siRNA (A). Phospho-GSK-3β (Ser9) Antibody #9336 was used to confirm downstream pathway inhibition (B).