

Akt1 siRNA (h): sc-29195

BACKGROUND

The serine/threonine kinase Akt family contains several members, including Akt1 (also designated PKB or RacPK), Akt2 (also designated PKB β or RacPK- β) and Akt 3 (also designated PKB γ or thymoma viral proto-oncogene 3), which exhibit sequence homology with the protein kinase A and C families and are encoded by the c-Akt proto-oncogene. All members of the Akt family have a pleckstrin homology domain. Akt1 and Akt2 are activated by PDGF stimulation. Activation is dependent on PDGFR- β Tyr residues 740 and 751, which bind the subunit of the phosphatidylinositol 3-kinase (PI 3-kinase) complex. Activation of Akt1 by Insulin or Insulin-growth factor-1 (IGF-1) results in phosphorylation of both Thr 308 and Ser 473. Phosphorylation of both residues is important to generate a high level of Akt1 activity. The phosphorylation of Thr 308 is not dependent on phosphorylation of Ser 473 *in vivo*. Thus, Akt proteins become phosphorylated and activated in Insulin/IGF-1-stimulated cells by an upstream kinase(s). The activation of Akt1 and Akt2 is inhibited by the PI kinase inhibitor wortmannin, suggesting that the protein signals downstream of the PI kinases.

CHROMOSOMAL LOCATION

Genetic locus: AKT1 (human) mapping to 14q32.33.

PRODUCT

Akt1 siRNA (h) is a target-specific 19-25 nt siRNA 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 Akt1 shRNA Plasmid (h): sc-29195-SH and Akt1 shRNA (h) Lentiviral Particles: sc-29195-V as alternate gene silencing products.

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

Akt1 siRNA (h) is recommended for the inhibition of Akt1 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.

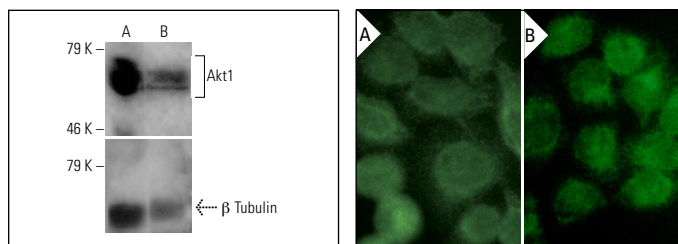
GENE EXPRESSION MONITORING

Akt1 (G-5): sc-55523 is recommended as a control antibody for monitoring of Akt1 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).

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor Akt1 gene expression knockdown using RT-PCR Primer: Akt1 (h)-PR: sc-29195-PR (20 μ l, 369 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

DATA



Akt1 siRNA (h): sc-29195. Western blot analysis of Akt1 expression in non-transfected control (A) and Akt1 siRNA double transfected (B) MCF7 cells. Blot probed with Akt1 (B-1): sc-5298, β Tubulin (D-10): sc-5274 used as specificity and loading control.

Akt1 siRNA (h): sc-29195. Immunofluorescence staining of methanol-fixed, control HeLa (A) and Akt1 siRNA silenced HeLa (B) cells showing diminished cytoplasmic and nuclear staining in the siRNA silenced cells. Cells probed with Akt1/2 (H-136): sc-8312.

SELECT PRODUCT CITATIONS

- Xin, M., et al. 2005. Nicotine inactivation of the proapoptotic function of bax through phosphorylation. *J. Biol. Chem.* 280: 10781-10789.
- Othman, E.M., et al. 2014. Signaling steps in the induction of genomic damage by Insulin in colon and kidney cells. *Free Radic. Biol. Med.* 68: 247-257.
- Tsai, J.P., et al. 2015. Licochalcone A induces autophagy through PI3K/Akt/mTOR inactivation and autophagy suppression enhances Licochalcone A-induced apoptosis of human cervical cancer cells. *Oncotarget* 6: 28851-28866.
- Ko, J.C., et al. 2016. Salinomycin enhances cisplatin-induced cytotoxicity in human lung cancer cells via down-regulation of Akt-dependent thymidylate synthase expression. *Biochem. Pharmacol.* 122: 90-98.
- Tung, C.L., et al. 2017. Salinomycin acts through reducing Akt-dependent thymidylate synthase expression to enhance erlotinib-induced cytotoxicity in human lung cancer cells. *Exp. Cell Res.* 357: 59-66.
- Kim, E.J., et al. 2018. Over-activation of Akt signaling leading to 5-fluorouracil resistance in SNU-C5/5-FU cells. *Oncotarget* 9: 19911-19928.

RESEARCH USE

For research use only, not for use in diagnostic procedures.