

Akt1/2 siRNA (h): sc-43609

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. This activation is dependent on PDGFR- β tyrosine 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, and 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. Taken together, this data strongly suggests that the protein signals downstream of the PI kinases.

REFERENCES

1. Burgering, B.M., et al. 1995. Protein kinase B (c-Akt) in phosphatidylinositol-3-OH kinase signal transduction. *Nature* 376: 599-602.
2. Datta, K., et al. 1995. AH/PH domain-mediated interaction between Akt molecules and its potential role in Akt regulation. *Mol. Cell. Biol.* 15: 2304-2310.

CHROMOSOMAL LOCATION

Genetic locus: AKT1 (human) mapping to 14q32.33, AKT2 (human) mapping to 19q13.2.

PRODUCT

Akt1/2 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 Akt1/2 shRNA Plasmid (h): sc-43609-SH and Akt1/2 shRNA (h) Lentiviral Particles: sc-43609-V as alternate gene silencing products.

STORAGE AND RESUSPENSION

Store lyophilized siRNA duplex at -20 $^{\circ}$ C with desiccant. Stable for at least one year from the date of shipment. Once resuspended, store at -20 $^{\circ}$ 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

Akt1/2 siRNA (h) is recommended for the inhibition of Akt1 and Akt2 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 (B-1): sc-5298 is recommended as a control antibody for monitoring of Akt1/2 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 and Akt2 gene expression knockdown using RT-PCR Primer: Akt1/2 (h)-PR: sc-43609-PR (20 μ l, 455 bp). Annealing temperature for the primers should be 55-60 $^{\circ}$ C and the extension temperature should be 68-72 $^{\circ}$ C.

SELECT PRODUCT CITATIONS

1. Liu, H., et al. 2011. Hepatitis B virus large surface antigen promotes liver carcinogenesis by activating the Src/PI3K/Akt pathway. *Cancer Res.* 71: 7547-7557.
2. Nikhil, K., et al. 2014. Pterostilbene-isothiocyanate conjugate suppresses growth of prostate cancer cells irrespective of androgen receptor status. *PLoS ONE* 9: e93335.
3. Zhu, G.C., et al. 2015. Metadherin regulation of vascular endothelial growth factor expression is dependent upon the PI3K/Akt pathway in squamous cell carcinoma of the head and neck. *Medicine* 94: e502.
4. Skrypek, N., et al. 2015. The oncogenic receptor ErbB2 modulates gemcitabine and irinotecan/SN-38 chemoresistance of human pancreatic cancer cells via hCNT1 transporter and multidrug-resistance associated protein MRP-2. *Oncotarget* 6: 10853-10867.
5. Caino, M.C., et al. 2015. PI3K therapy reprograms mitochondrial trafficking to fuel tumor cell invasion. *Proc. Natl. Acad. Sci. USA* 112: 8638-8643.
6. Vétillard, A., et al. 2015. Akt inhibition improves irinotecan treatment and prevents cell emergence by switching the senescence response to apoptosis. *Oncotarget* 6: 43342-43362.
7. Polimeni, M., et al. 2016. Multi-walled carbon nanotubes directly induce epithelial-mesenchymal transition in human bronchial epithelial cells via the TGF- β -mediated Akt/GSK-3/SNAIL-1 signalling pathway. *Part. Fibre Toxicol.* 13: 27.

RESEARCH USE

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