

Akt1 siRNA (r): sc-108059

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 (rat) mapping to 6q32.

PRODUCT

Akt1 siRNA (r) is a pool of 3 target-specific 19-25 nt siRNAs 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 (r): sc-108059-SH and Akt1 shRNA (r) Lentiviral Particles: sc-108059-V as alternate gene silencing products.

For independent verification of Akt1 (r) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-108059A, sc-108059B and sc-108059C.

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 (r) is recommended for the inhibition of Akt1 expression in rat cells.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.

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 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 (r)-PR: sc-108059-PR (20 μ l, 560 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

- Grzesik, B.A., et al. 2013. Efficient gene delivery to primary alveolar epithelial cells by nucleofection. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 305: L786-L794.
- Meng, X., et al. 2014. Notoginsenoside R1-mediated neuroprotection involves estrogen receptor-dependent crosstalk between Akt and ERK1/2 pathways: a novel mechanism of Nrf2/ARE signaling activation. *Free Radic. Res.* 48: 445-460.
- Than, A., et al. 2015. Apelin enhances brown adipogenesis and browning of white adipocytes. *J. Biol. Chem.* 290: 14679-14691.
- Xia, B., et al. 2015. Akt enhances nerve growth factor-induced axon growth via activating the Nrf2/ARE pathway. *Int. J. Mol. Med.* 36: 1426-1432.
- Gao, Y., et al. 2015. Differential IKK/NF κ B activity is mediated by TSC2 through mTORC1 in PTEN-null prostate cancer and tuberous sclerosis complex tumor cells. *Mol. Cancer Res.* 13: 1602-1614.
- Lei, S., et al. 2019. Hyperglycemia-induced oxidative stress abrogates remifentanyl preconditioning-mediated cardioprotection in diabetic rats by impairing caveolin-3-modulated PI3K/Akt and JAK2/Stat3 signaling. *Oxid. Med. Cell. Longev.* 2019: 9836302.
- Wang, X., et al. 2020. A cathelicidin-related antimicrobial peptide suppresses cardiac hypertrophy induced by pressure overload by regulating IGF1R1/PI3K/Akt and TLR9/AMPK α . *Cell Death Dis.* 11: 96.

RESEARCH USE

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