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# Akt1 shRNA (h2) Lentiviral Particles: sc-44198-V

## BACKGROUND

The serine/threonine kinase Akt family contains several members, including Akt1 (also designated PKB or RacPK), Akt2 (also designated PKB $\beta$  or RacPK- $\beta$ ) and Akt3 (also designated PKB $\gamma$  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- $\beta$  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-I) 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-I-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.

## REFERENCES

- Burgering, B.M., et al. 1995. Protein kinase B (c-Akt) in phosphatidylinositol 3-OH kinase signal transduction. *Nature* 376: 599-602.
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- Franke, T.F., et al. 1995. The protein kinase encoded by the Akt proto-oncogene is a target of the PDGF-activated phosphatidylinositol 3-kinase. *Cell* 81: 727-736.
- Cheng, J.Q., et al. 1996. Amplification of Akt2 in human pancreatic cancer cells and inhibition of Akt2 expression and tumorigenicity by antisense RNA. *Proc. Natl. Acad. Sci. USA* 93: 3636-3641.
- Barthel, A., et al. 1998. Protein kinase C modulates the Insulin-stimulated increase in Akt1 and Akt3 activity in 3T3-L1 adipocytes. *Biochem. Biophys. Res. Commun.* 243: 509-513.

## CHROMOSOMAL LOCATION

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

## PRODUCT

Akt1 shRNA (h2) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200  $\mu$ l frozen stock containing  $1.0 \times 10^6$  infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see Akt1 siRNA (h2): sc-44198 and Akt1 shRNA Plasmid (h2): sc-44198-SH as alternate gene silencing products.

## STORAGE

Store lentiviral particles at  $-80^\circ\text{C}$ . Stable for at least one year from the date of shipment. Once thawed, particles can be stored at  $4^\circ\text{C}$  for up to one week. Avoid repeated freeze thaw cycles.

## APPLICATIONS

Akt1 shRNA (h2) Lentiviral Particles is recommended for the inhibition of Akt1 expression in human cells.

## SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200  $\mu$ l frozen viral stock containing  $1.0 \times 10^6$  infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

## GENE EXPRESSION MONITORING

Akt1 (A-11): sc-377457 is recommended as a control antibody for monitoring of 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).

To ensure optimal results, the following support (secondary) reagents are recommended: 1) Western Blotting: use goat anti-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker™ compatible goat anti-mouse IgG-HRP: sc-2031 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

## RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor Akt1 gene expression knockdown using RT-PCR Primer: Akt1 (h2)-PR: sc-44198-PR (20  $\mu$ l, 515 bp). Annealing temperature for the primers should be  $55-60^\circ\text{C}$  and the extension temperature should be  $68-72^\circ\text{C}$ .

## BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

## RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.

## PROTOCOLS

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