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SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

[linkedin.com/company/szaboscandic](https://www.linkedin.com/company/szaboscandic) 

Akt2 siRNA (h2): sc-44245

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 Akt3 (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 that is dependent on PDGFR- β tyrosine residues 740 and 751, which bind the subunit of the phosphatidylinositol 3-kinase (PI 3-kinase) complex. Akt proteins become phosphorylated and activated in Insulin/IGF-1-stimulated cells by an upstream kinase(s), and 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. Akt3 is phosphorylated on a serine residue in response to Insulin, and this activation is inhibited by prior activation of protein kinase C. Akt3 is expressed in 3T3-L1 fibroblasts, adipocytes and skeletal muscle and may be involved in various biological processes, including adipocyte and muscle differentiation, glycogen synthesis, glucose uptake, apoptosis and cellular proliferation.

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.
3. 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.
4. 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.
5. 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: AKT2 (human) mapping to 19q13.2.

PRODUCT

Akt2 siRNA (h2) 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 Akt2 shRNA Plasmid (h2): sc-44245-SH and Akt2 shRNA (h2) Lentiviral Particles: sc-44245-V as alternate gene silencing products.

For independent verification of Akt2 (h2) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-44245A, sc-44245B and sc-44245C.

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

Akt2 shRNA Plasmid (h2) is recommended for the inhibition of 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

Akt2 (F-7): sc-5270 is recommended as a control antibody for monitoring of Akt2 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 reagents are recommended: 1) Western Blotting: use m-IgG κ BP-HRP: sc-516102 or m-IgG κ BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker[™] Molecular Weight Standards: sc-2035, UltraCruz[®] Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-IgG κ BP-FITC: sc-516140 or m-IgG κ BP-PE: sc-516141 (dilution range: 1:50-1:200) with UltraCruz[®] Mounting Medium: sc-24941 or UltraCruz[®] Hard-set Mounting Medium: sc-359850.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor Akt2 gene expression knockdown using RT-PCR Primer: Akt2 (h2)-PR: sc-44245-PR (20 μ l, 495 bp). Annealing temperature for the primers should be 55-60 $^{\circ}$ C and the extension temperature should be 68-72 $^{\circ}$ C.

RESEARCH USE

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

PROTOCOLS

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