

Akt1 (h): 293T Lysate: sc-158248

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. 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-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.
- 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.
- 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.
- Nakatani, K., et al. 1999. Identification of a human Akt3 (protein kinase B γ) which contains the regulatory serine phosphorylation site. *Biochem. Biophys. Res. Commun.* 257: 906-910.
- Turinsky, J., et al. 1999. Akt kinases and 2-deoxyglucose uptake in rat skeletal muscles *in vivo*: study with Insulin and exercise. *Am. J. Physiol.* 276: R277-R282.
- Murthy, S.S., et al. 2000. Mapping of Akt3, encoding a member of the Akt/protein kinase B family, to human and rodent chromosomes by fluorescence *in situ* hybridization. *Cytogenet. Cell Genet.* 88: 38-40.

CHROMOSOMAL LOCATION

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

PRODUCT

Akt1 (h): 293T Lysate represents a lysate of human Akt1 transfected 293T cells and is provided as 100 μ g protein in 200 μ l SDS-PAGE buffer.

APPLICATIONS

Akt1 (h): 293T Lysate is suitable as a Western Blotting positive control for human reactive Akt1 antibodies. Recommended use: 10-20 μ l per lane.

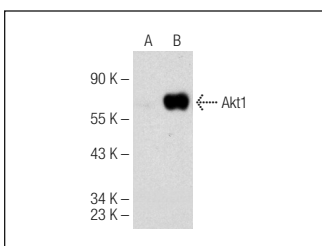
Control 293T Lysate: sc-117752 is available as a Western Blotting negative control lysate derived from non-transfected 293T cells.

Akt1 (A-11): sc-377457 is recommended as a positive control antibody for Western Blot analysis of enhanced human Akt1 expression in Akt1 transfected 293T cells (starting dilution 1:100, dilution range 1:100-1:1,000).

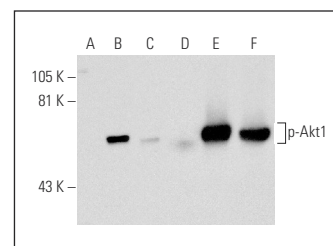
RECOMMENDED SUPPORT REAGENTS

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.

DATA



Akt1 (A-11): sc-377457. Western blot analysis of Akt1 expression in non-transfected: sc-117752 (A) and human Akt1 transfected: sc-158248 (B) 293T whole cell lysates.



Western blot analysis of Akt1 phosphorylation in non-transfected: sc-117752 (A,D), untreated human Akt1 transfected: sc-158248 (B,E) and lambda protein phosphatase (sc-200312A) treated human Akt1 transfected: sc-158248 (C,F) 293T whole cell lysates. Antibodies tested include p-Akt1 (Thr 308): sc-135650 (A,B,C) and Akt1 (C-20): sc-1618 (D,E,F).

STORAGE

Store at -20° C. Repeated freezing and thawing should be minimized. Sample vial should be boiled once prior to use. Non-hazardous. No MSDS required.

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.