SANTA CRUZ BIOTECHNOLOGY, INC.

p-Akt1/2/3 (Tyr 315/316/312): sc-293095

SANTA CRUZ BIOTECHNOLOGY

The Power to Question

BACKGROUND

The serine/threonine kinase Akt family contains several members, including Akt1 (also designated PKB or RacPK), Akt2 (also designated PKBβ or RacPK-β) and Akt 3 (also designated PKBγ or thyoma 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. 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. However, the activation of Akt3 by Insulin is inhibited by prior activation of protein kinase C via a mechanism that does not require the presence of the PH domain. 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.

SOURCE

p-Akt1/2/3 (Tyr 315/316/312) is a rabbit polyclonal antibody raised against a short amino acid sequence containing Tyr 315/316/312 phosphorylated Akt1/2/3 of human origin.

PRODUCT

Each vial contains 100 µg IgG in 1.0 ml PBS with < 0.1% sodium azide and 0.1% gelatin.

STORAGE

Store at 4° C, **DO NOT FREEZE**. Stable for one year from the date of shipment. Non-hazardous. No MSDS required.

APPLICATIONS

p-Akt1/2/3 (Tyr 315/316/312) is recommended for detection of Tyr 315 phosphorylated Akt1, correspondingly Tyr 316 phosphorylated Akt2 and correspondingly Tyr 312 phosphorylated Akt3 of mouse, rat and human origin by Western Blotting (starting dilution 1:200, dilution range 1:100-1:1000), immunoprecipitation [1-2 µg per 100-500 µg of total protein (1 ml of cell lysate)], immunofluorescence (starting dilution 1:50, dilution range 1:50-1:500) and immunohistochemistry (including paraffin-embedded sections) (starting dilution 1:50, dilution range 1:50-1:500).

Molecular Weight of Akt1: 62 kDa.

Molecular Weight of Akt2: 56 kDa.

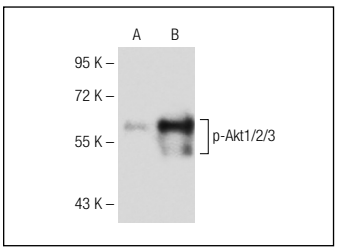
Molecular Weight of Akt3: 62 kDa.

Positive Controls: HeLa whole cell lysate: sc-2200, Hep G2 cell lysate: sc-2227 or human breast carcinoma tissue.

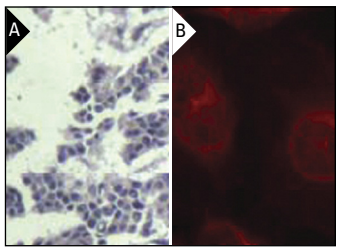
RECOMMENDED SECONDARY REAGENTS

To ensure optimal results, the following support (secondary) reagents are recommended: 1) Western Blotting: use goat anti-rabbit IgG-HRP: sc-2004 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible goat anti-rabbit IgG-HRP: sc-2030 (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) Immunoprecipitation: use Protein A/G PLUS-Agarose: sc-2003 (0.5 ml agarose/2.0 ml). 3) Immunofluorescence: use goat anti-rabbit IgG-FITC: sc-2012 (dilution range: 1:100-1:400) or goat anti-rabbit IgG-TR: sc-2780 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941. 4) Immunohistochemistry: use ImmunoCruz™: sc-2051 or ABC: sc-2018 rabbit IgG Staining Systems.

DATA



p-Akt1/2/3 (Tyr 315/316/312): sc-293095. Western blot analysis of Akt1/2/3 phosphorylation expression in untreated (**A**) and EGF treated (**B**) Hep G2 whole cell lysates.



p-Akt1/2/3 (Tyr 315/316/312): sc-293095. Immunoperoxidase staining of formalin-fixed, paraffin-embedded human breast carcinoma tissue (**A**) and immunofluorescence staining of methanol-fixed HeLa cells (**B**) showing nuclear and cytoplasmic localization.

RESEARCH USE

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

PROTOCOLS

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