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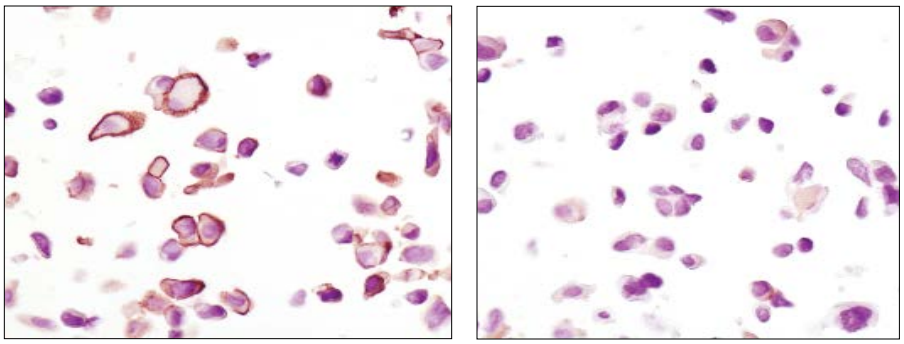


Immunohistochemical analysis of paraffin-embedded LNCaP cells, untreated (left) or treated with LY294002 (right) using Phospho-Akt (Ser473) (D9E) Rabbit mAb #4060.

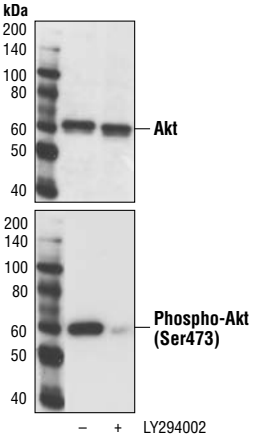
Background: Akt, also referred to as PKB or Rac, plays a critical role in controlling survival and apoptosis (1-3). This protein kinase is activated by insulin and various growth and survival factors and functions in a wortmannin-sensitive pathway involving PI3 kinase (2,3). Akt is activated by phospholipid binding and activation loop phosphorylation at Thr308 by PDK1 (4) and by phosphorylation within the carboxy terminus at Ser473. The previously elusive PDK2 responsible for phosphorylation of Akt at Ser473 has been identified as mammalian target of rapamycin (mTor) in a rapamycin-insensitive complex with Rictor and Sin1 (5,6). Akt promotes cell survival by inhibiting apoptosis through its ability to phosphorylate and inactivate several targets, including Bad (7), Forkhead transcription factors (8), c-Raf (9) and caspase-9. PTEN phosphatase is a major negative regulator of the PI3 kinase/Akt signaling pathway (10). LY294002 is a specific PI3 kinase inhibitor (11).

One of the essential functions of Akt is the regulation of glycogen synthesis through phosphorylation and inactivation of GSK-3α and β (12,13). Akt may also play a role in insulin stimulation of glucose transport (12).

In addition to its role in survival and glycogen synthesis, Akt is involved in cell cycle regulation by preventing GSK-3β mediated phosphorylation and degradation of cyclin D1 (14) and by negatively regulating the cyclin dependent kinase inhibitors p27 Kip (15) and p21 Waf1 (16). Akt also plays a critical role in cell growth by directly phosphorylating mTOR in a rapamycin-sensitive complex containing raptor (17). More importantly, Akt phosphorylates and inactivates tuberin (TSC2), an inhibitor of mTOR within the mTOR-raptor complex (18). Inhibition of mTOR stops the protein synthesis machinery due to inactivation of its effector, p70 S6 kinase and activation of the eukaryotic initiation factor, 4E binding protein 1 (4E-EP1), an inhibitor of translation (19,20).

Description: Each control slide contains formalin fixed, paraffin-embedded LNCaP cells, both untreated and treated with PI3-Kinase inhibitor LY294002, that serve as a control for Phospho-Akt (Ser473) immunostaining. Western blot analysis was performed on extracts derived from the same cells to verify the efficacy of the LY942002 treatment.

Applications: These slides are intended for use in immunohistochemical assays.



◀ *Western blot analysis of extracts from LNCaP cells, untreated or treated with LY294002, using Akt Antibody #9272 (upper) or Phospho-Akt (Ser 473) (193H121) Rabbit mAb #4058 (lower). This assay serves as a control for the efficacy of the LY94002 treatment.*

Entrez-Gene ID # 207
Swiss-Prot Acc. # P31749

Storage: Store at 4° C.

Optimal staining is achieved if slides are stained following CST's standard IHC protocols and are used within 8 weeks of assay date; however, signals may persist beyond two months.

For application specific protocols please see the web page for this product at www.cellsignal.com.

Please visit www.cellsignal.com for a complete listing of recommended companion products.

Background References:

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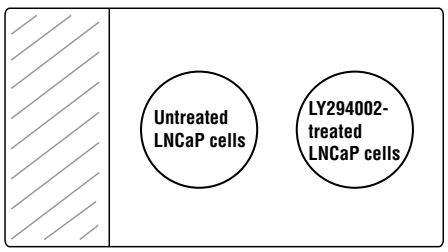

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Applications Key: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide
Species Cross-Reactivity Key: **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% sequence homology.