

A Strategy for Survival: The Akt Signaling Cascade

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In the past decade, there has been a surge of research aimed at unraveling the various pathways of apoptosis. However, insight into survival pathways promises to prove equally important for our future understanding of the delicate balance between life and death of the cell. One of the key enzymes for regulating anti-apoptotic events is the protein kinase Akt. Understanding the signaling cascade mediated by this enzyme should expand our knowledge of cellular life and death.

INTRODUCTION

Akt, also known as protein kinase B (PKB) or RAC-PK (a protein kinase related to protein kinases A and C), is emerging as a key player in the survival of many cell types (1,2). It is a serine-threonine kinase involved in signaling cascades regulating anti-apoptotic events (3). As recent experimental evidence suggests, Akt plays an important role in the pathogenesis of degenerative diseases and cancer (4) and this is taking center stage in cellular research. The schematic shown in Figure 1 illustrates some of the key events linked to the regulation of apoptosis and survival.

AKT SUBSTRATES

Numerous Akt substrates have been reported in recent years. First identified was the Bcl-2 family member, Bad, a pro-apoptotic protein that binds and inhibits anti-apoptotic Bcl-2 molecules when Bad is not phosphorylated (5). Other direct targets of Akt are members of the Forkhead-related family of mammalian transcription factors that, when phosphorylated, are retained in the cytoplasm and thereby are prevented from affecting transcription (6). Another notable substrate of Akt is the death protease caspase-9 (7). Phosphorylation of caspase-9 decreases apoptosis by directly inhibiting the protease activity.

Upstream kinases are involved in the activation of Akt by phosphorylating the enzyme at a serine residue (S⁴⁷³) near the C-terminus. Phosphorylation of S⁴⁷³ is critical for the regulation of Akt and for maintenance of its activity. Although Akt activation can be measured by examining its kinase activity on exogenous substrates, specific antibodies directed against the primary phosphorylation sites of Akt are extremely helpful because they can be used for Western analysis and immunolocalization of activated Akt.

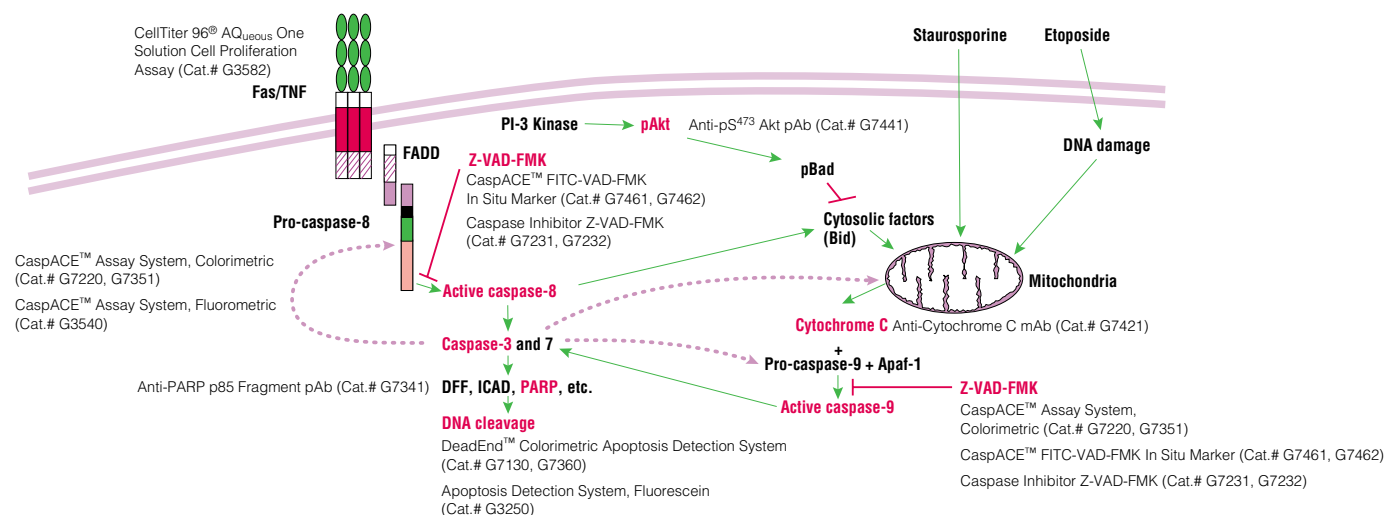


Figure 1. An apoptosis/survival model system. This schematic diagram illustrates the key role that Akt plays in anti-apoptotic events. Relevant Promega products are noted. The figure is a modification of one that originally appeared in Sun, X.M. *et al.* (1999) *J. Biol. Chem.* **274**, 5053.

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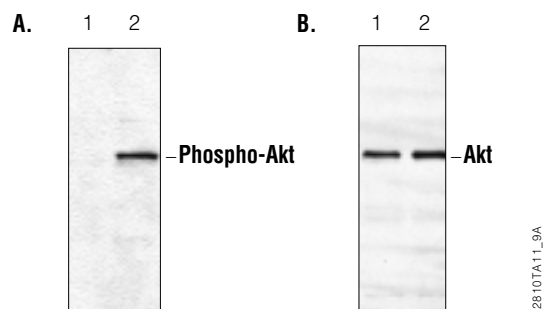


Figure 2. Detection of phosphorylated Akt by Western blot analysis with Anti-pS⁴⁷³ Akt pAb. **Panel A:** NIH3T3 total cell extract (10µg per lane) was resolved by polyacrylamide gel electrophoresis and blotted onto nitrocellulose. Lane 1, untreated cells; lane 2, cells pretreated with PDGF (Life Technologies, Inc.) at 50ng/ml for 20 minutes. Anti-pS⁴⁷³ Akt pAb (Cat.# G7441) was used at a 1:2,500 dilution. The blot was probed with Donkey Anti-Rabbit IgG (H+L), HRP, Anti-ACTIVE® Qualified pAb (Cat.# V7951) at 1:10,000 dilution followed by chemiluminescent detection. **Panel B:** A pan-Akt pAb (New England Biolabs) reveals total Akt in both stimulated and unstimulated NIH3T3 cell extracts. Secondary antibody and detection method were the same as those used in Panel A.

A NEW AKT-SPECIFIC ANTIBODY

Figure 2, Panel A, illustrates the selective and specific binding of Promega's new Anti-pS⁴⁷³ Akt pAb (Cat.# G7441) in PDGF-stimulated mouse NIH3T3 cell lysates. No detection is observed in lysates from unstimulated cells, in which Akt is not phosphorylated. In contrast, interrogation with a pan-Akt pAb (Figure 2, Panel B) reveals the presence of total Akt in both of these cell lysates. Unlike other phosphorylation-specific Akt antibodies, Promega's Anti-pS⁴⁷³ Akt pAb also can be used for immunostaining. Figure 3, Panels A and B, illustrate antibody labeling in human Jurkat cells and primary rat neurospheres, respectively. In addition to showing the exquisite specificity of this antibody for the Akt phosphorylation domain, these data demonstrate the advantageous species cross-reactivity of Anti-pS⁴⁷³ Akt pAb.

SUMMARY

Promega provides a range of reagents for studying discrete events that regulate apoptosis and survival. Our new phospho-specific antibody, Anti-pS⁴⁷³ Akt pAb, can be used to gain new insights into cellular survival pathways.

To facilitate the study of phosphorylated caspase-9, Promega is developing an anti-caspase-9 pAb. For information on this product in development, please contact Pam Guthmiller, Product Manager; pguthmil@promega.com.

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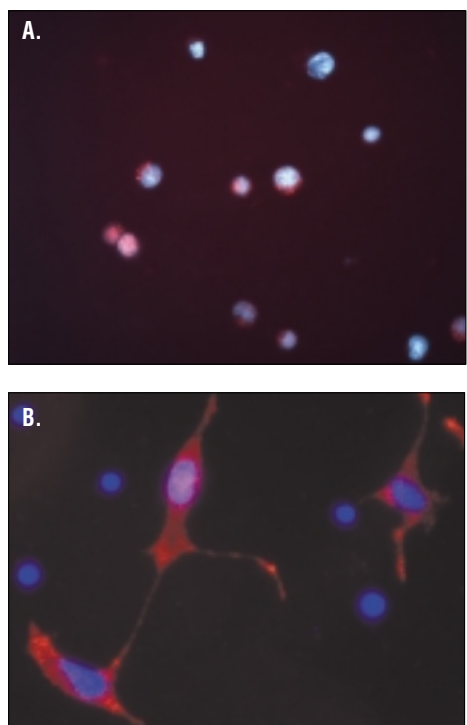


Figure 3. Immunocytochemical staining of phosphorylated Akt. **Panel A:** Human Jurkat cells were collected, washed and fixed onto poly-L-lysine-coated slides with 4% paraformaldehyde for 30 minutes. Prior to staining, cells were permeabilized with 0.2% Triton® X-100 and blocked with 5% normal donkey serum. Anti-pS⁴⁷³ Akt pAb (Cat.# G7441) was used at a 1:50 dilution. Positive cells were visualized using a donkey anti-rabbit, Cy[™]3-conjugated secondary antibody (Jackson ImmunoResearch) at a 1:500 dilution. Nuclei were stained using DAPI. **Panel B:** Embryonic (day 17) rat brain cells were collected, grown in NB 27 medium with 20ng/ml EGF and FGF and plated onto Lab-Tek® II chamber slides coated with EHS. The fixed cells were then treated as in Panel A.

Ordering Information

Product	Size	Cat.#
Anti-pS ⁴⁷³ Akt pAb	40µl	G7441
Donkey Anti-Rabbit IgG (H+L), HRP, Anti-ACTIVE® Qualified pAb	60µl	V7951
Anti-Cytochrome C mAb	100µg	G7421
rhEGF	100µg	G5021
rhFGF, Basic	25µg	G5071
EHS Cell Attachment Matrix	5ml	G5971

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