

# Anti-phospho-Akt1/PKB $\alpha$ (Ser473)

Polyclonal Antibody

Cat. # 07-310

Lot # DAM1536595

pack size: 200  $\mu$ g

Store at -20°C

FOR RESEARCH USE ONLY  
NOT FOR USE IN HUMANS



## Certificate of Analysis

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| Applications | Species Cross-Reactivity | Antibody Isotype | Epitope/Region | Host Species | Molecular Weight | Accession #  |
|--------------|--------------------------|------------------|----------------|--------------|------------------|--------------|
| WB           | H, M                     | IgG              | N/A            | Rb           | 60 kDa           | NP_001014431 |

### Background

The serine threonine protein kinase encoded by the AKT1 gene is catalytically inactive in serum starved primary and immortalized fibroblasts. AKT1 and the related AKT2 are activated by platelet derived growth factor. The activation is rapid and specific. In the developing nervous system AKT is a critical mediator of growth factor induced neuronal survival. Survival factors can suppress apoptosis in a transcription independent manner by activating the serine/threonine kinase AKT1, which then phosphorylates and inactivates components of the apoptotic machinery. Multiple alternatively spliced transcript variants have been found for this gene (referenced from entrez gene).

### Presentation

Purified rabbit polyclonal IgG in buffer containing 0.07 M Tris-glycine, pH 7.4, 0.105 M NaCl, 0.035% sodium azide and 30% glycerol. Store at -20°C.

### Concentration

0.25 mg/mL

### Specificity

Recognizes phosphorylated Akt1/PKB $\alpha$ , Mr 60 kDa.

### Species Cross-reactivity

Human and mouse. Predicted cross-reactivity with rat, bovine, chicken, and *Xenopus* based on immunogen sequence homology.

### Immunogen

KLH-conjugated, synthetic peptide containing a pSer that corresponds to amino acid position 473 of human Akt1. The immunizing sequence is identical in mouse and bovine Akt1. Akt2 and Akt3 share significant homology with the peptide immunogen sequence.

### Molecular Weight

60 kDa

### Method of Purification

Protein A Purified

### Storage and Handling

Stable for 1 year at -20°C from date of receipt.

Handling Recommendations: Upon first thaw, and prior to removing the cap, centrifuge the vial and gently mix the solution. Aliquot into microcentrifuge tubes and store at -20°C. Avoid repeated freeze/thaw cycles, which may damage IgG and affect product performance. Note: Variability in freezer temperatures below -20°C may cause glycerol containing solutions to become frozen during storage.

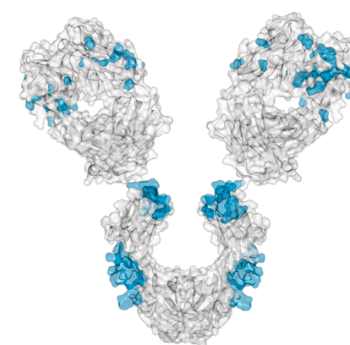
### Control

PDGF-treated 3T3/NIH cells.

### Quality Control Testing

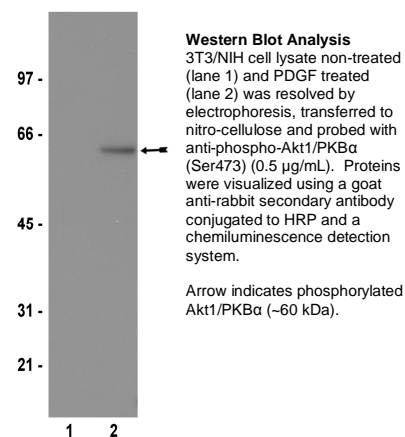
routinely evaluated by western blot on lysates from mouse NIH-3T3 fibroblasts treated with PDGF.

**Western Blot Analysis:** 0.5-2  $\mu$ g/mL of this lot detected phosphorylated Akt1/PKB $\alpha$  in lysates from mouse 3T3/NIH fibroblasts treated with 100 ng/mL PDGF for 20 minutes.



### References

- Butler, B., et al. (2003). *J. Biol. Chem.* 278: 5264-5270.
- Ogihara, T., et al. (2001). *Diabetes.* 50: 573-583.
- Alessi, D.R., et al. (1998). *Curr. Biol.* 8: 69-81.
- Cohen, P., et al. (1997). *FEBS Lett.* 410: 3-10.
- James, S.R., et al. (1996). *Biochem. J.* 315: 709-713.



**APPLICATION LEGEND:** WB Western Blotting IP Immunoprecipitation IC Immunocytochemistry IF Immunofluorescence  
IH Immunohistochemistry (Tissue)

**SPECIES LEGEND:** H Human M Mouse R Rat Rb Rabbit WR Most Common Vertebrates

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**PROTOCOL****Western Blot**

1. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on a cell lysate sample (cell lysis buffer: 50 mM Tris-HCl, pH 7.4; 1% NP-40; 0.25% sodium deoxycholate; 150 mM NaCl; 1 mM EDTA; 1 mM PMSF; 1  $\mu$ g/mL each aprotinin, leupeptin, pepstatin; 1 mM Na<sub>3</sub>VO<sub>4</sub>; 1 mM NaF) and transfer the proteins to nitrocellulose. Wash the blotted nitrocellulose twice with water.
2. Block the blotted nitrocellulose in freshly prepared TBS containing 5% nonfat dry milk (Catalog # 20-200) and 0.05% Tween 20 (TBST-MLK) for 2 hours at room temperature with constant agitation.
3. Incubate the nitrocellulose with **0.5-2  $\mu$ g/mL of anti-phospho-Akt1/PKB $\alpha$  (Ser473)**, diluted in freshly prepared TBST-MLK for 1.5 hours with agitation at room temperature.
4. Wash the nitrocellulose three times with water.
5. Incubate the nitrocellulose in the secondary reagent of choice (a goat anti-rabbit HRP conjugated IgG, Catalog # 12-348, 1:5000 dilution was used) in TBST-MLK for 1.5 hours at room temperature with agitation.
6. Rinse the nitrocellulose with water twice.
7. Wash the nitrocellulose in TBS-0.05% Tween 20 for 3-5 minutes.
8. Wash the nitrocellulose three times with water.
9. Use detection method of choice (enhanced chemiluminescence was used).

**RELATED PRODUCTS (specific)**

| cat #  | description   |
|--------|---|
| 16-293 | ■ Anti-Akt/PKB, PH Domain, clone SKB1, Alexa Fluor® 488 conjugate                 |
| 16-294 | ■ Anti-Akt/PKB, PH Domain, clone SKB1, Alexa Fluor® 555 conjugate                 |
| AB3137 | ■ Anti-Akt1   |
| AB3132 | ■ Anti-Akt1, phospho-specific (Ser473)  |
| 07-416 | ■ Anti-Akt1/PKB $\alpha$  |
| 05-796 | ■ Anti-Akt1/PKB $\alpha$ , clone AW24   |
| 06-885 | ■ Anti-Akt1/PKB $\alpha$ , PH domain, polyclonal                                  |
| 07-789 | ■ Anti-phospho-AKT (Thr34)  |
| 07-310 | ■ Anti-phospho-Akt1/PKB $\alpha$ (Ser473)   |
| 05-669 | ■ Anti-phospho-Akt1/PKB $\alpha$ (Ser473), clone 11E6                             |
| 16-305 | ■ Anti-phospho-Akt1/PKB $\alpha$ (Ser473), clone 11E6, Alexa Fluor® 488 conjugate |
| 16-306 | ■ Anti-phospho-Akt1/PKB $\alpha$ (Ser473), clone 11E6, Alexa Fluor® 555 conjugate |
| 05-736 | ■ Anti-phospho-Akt1/PKB $\alpha$ (Ser473), clone SK703                            |
| 06-678 | ■ Anti-phospho-Akt1/PKB $\alpha$ (Thr308)   |
| 05-802 | ■ Anti-phospho-Akt1/PKB $\alpha$ (Thr308), clone NL50                             |
| 14-453 | ■ Akt1/PKB $\alpha$ ( $\Delta$ PH, S473D), active                                 |
| 14-245 | ■ Akt1/PKB $\alpha$ , PH Domain (aa 1-149)  |
| 14-341 | ■ Akt1/PKB $\alpha$ , (PHD deletion), active                                      |
| 14-276 | ■ Akt1/PKB $\alpha$ , active  |
| 14-279 | ■ Akt1/PKB $\alpha$ , unactive  |
| 14-241 | ■ Akt1/PKB $\alpha$ -GST, unactivated agarose conjugate                           |
| 17-253 | ■ Akt1/PKB $\alpha$ cDNA (activated) Expression Kit                               |
| 17-252 | ■ Akt1/PKB $\alpha$ cDNA (dominant negative) Expression Kit                       |
| 21-151 | ■ Akt1/PKB $\alpha$ cDNA (activated) in pUSEamp                                   |
| 21-152 | ■ Akt1/PKB $\alpha$ cDNA (dominant negative) in pUSEamp                           |

**RELATED PRODUCTS (non-specific)**

| cat #       | description   |
|-------------|---|
| IPVH00010   | ■ Immobilon-P 26.5 cm x 3.75 m Roll PVDF 0.45 $\mu$ m     |
| IPFL00010   | ■ Immobilon-FL 26.5 cm x 3.75 m Roll PVDF 0.45 $\mu$ m    |
| IPVH07850   | ■ Immobilon-P 7 x 8.4 cm PVDF 0.45 mm (sheet) 50/pk       |
| ISEQ00010   | ■ Immobilon-P SQ 26.5 cm x 3.75 m 1 roll PVDF 0.2 $\mu$ m |
| ISEQ07850   | ■ Immobilon-P 7 x 8.4 cm PVDF 0.2 mm (sheet) 50/pk        |
| IPFL07810   | ■ Immobilon-FL 7 x 8.4 cm PVDF 0.45 mm (sheet) 10/pk      |
| WBKLS0100   | ■ Immobilon Western Chemilum HRP Substrate 100 mL         |
| 17-373      | ■ Spray & Glow™ ECL WB Detection System 1 ea              |
| 2060        | ■ Re-Blot Western Blot Recycling Kit                      |
| 2500        | ■ Re-Blot Plus Western Blot Recycling Kit                 |
| B2080-175GM | ■ Blot Quick Blocker Membrane Blocking Agent 175G         |

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