

Certificate of Analysis

Anti-phospho-Akt1/PKB α (Ser473)

(rabbit polyclonal IgG)

Catalog # 07-310

Lot # JBC1355778

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.

Specificity: Recognizes phosphorylated Akt1/PKB α , Mr 60 kDa.

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

Formulation: 200 μ g of protein A purified rabbit IgG in 200 μ L of buffer (0.07 M Tris-glycine, pH 7.4, 0.105 M NaCl, 0.035% sodium azide and 30% glycerol). Store at -20°C.

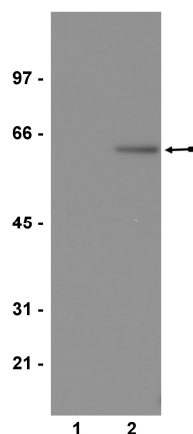
Storage and Stability: Stable for 2 years at -20°C from date of shipment.

Handling Recommendations: Upon receipt, 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.

**FOR RESEARCH USE ONLY
NOT FOR USE IN HUMANS**

Quality Control Testing

Immunoblot Analysis: 0.5-2 μ g/mL of this lot detected phosphorylated Akt1/PKB α in lysates from mouse 3T3/NIH fibroblasts treated with 100 ng/mL PDGF for 20 minutes.



Immunoblot Analysis

3T3/NIH cell lysate non-treated (lane 1) and PDGF treated (lane 2) was resolved by electrophoresis, transferred to nitrocellulose and probed with anti-phospho-Akt1/PKB α (Ser473) (0.5 μ g/mL). Proteins were visualized using a goat anti-rabbit secondary antibody conjugated to HRP and a chemiluminescence detection system. Arrow indicates phosphorylated Akt1/PKB α (~60 kDa).

Application References:

1. Butler, B., *et al.*, *J. Biol. Chem.* **278**: 5264-5270, 2003.
2. Ogihara, T., *et al.*, *Diabetes* **50**: 573-583, 2001..

General References:

3. Cross, D.A., *et al.*, *Nature* **378**: 785-789, 1995.
4. James, S.R., *et al.*, *Biochem. J.* **315**: 709-713, 1996.
5. Alessi, D.R., *et al.*, *Curr. Biol.* **8**: 69-81, 1998.
6. Alessi, D.R., *et al.*, *Curr. Biol.* **7**: 776-789, 1997.
7. Cohen, P., *et al.*, *FEBS Lett.* **410**: 3-10, 1997.

Immunoblot Protocol

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 µg/mL each aprotinin, leupeptin, pepstatin; 1 mM Na₃VO₄; 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 µg/mL of anti-phospho-Akt1/PKBα (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).