

Anti-phospho-Akt1/PKB α (Ser473)

(rabbit polyclonal IgG)

Catalog # 07-310

Lot # 28816

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 60kDa and an additional unknown band, Mr 90kDa.

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

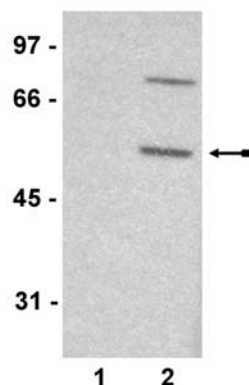
Formulation: 200 μ g of protein A purified rabbit IgG in 200 μ l of 0.1M Tris-glycine, pH 7.4, 0.15M NaCl, 0.05% sodium azide before the addition of glycerol to 30%. Liquid at -20°C.

Storage and Stability: Stable for 2 years at -20°C from date of shipment. For maximum recovery of product, centrifuge the vial prior to removing the cap.

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 in lysates from mouse NIH-3T3 fibroblasts treated with 100ng/ml PDGF for 20 minutes.



Immunoblot Analysis

Mouse NIH-3T3 cell lysate 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 (~60kDa). Lane 1: Non-treated 3T3 cell lysate. Lane 2: PDGF-treated 3T3 cell lysate.

Application References:

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

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: 50mM Tris-HCl, pH 7.4; 1% NP-40; 0.25% sodium deoxycholate; 150mM NaCl; 1mM EDTA; 1mM PMSF; 1 μ g/ml each aprotinin, leupeptin, pepstatin; 1mM Na₃VO₄; 1mM NaF) and transfer the proteins to nitrocellulose. Wash the blotted nitrocellulose twice with water.
2. Block the blotted nitrocellulose in freshly prepared 5% nonfat dry milk (Catalog # 20-200) in TBS with 0.05% Tween 20 (TBST-MLK) for 2 hours at room temperature with constant agitation.
3. Incubate the nitrocellulose with **0.5-2 μ g/ml of anti-phospho-Akt1/PKB α (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).