

Anti-phospho-Akt1/PKB α (Ser473), clone SK703

(rabbit monoclonal IgG)

Catalog # 05-736

Lot # 24657

Immunogen: KLH-conjugated, synthetic peptide containing a pSer that corresponds to amino acid position 473 of human Akt1/PKB α . 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. Cross-reactivity to phosphorylated Akt2 and Akt3 likely based on sequence homology.

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

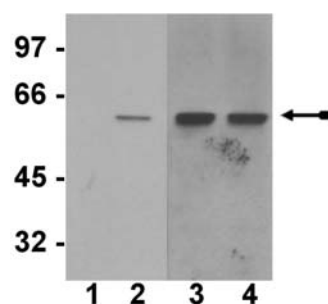
Formulation: 100 μ g of protein A purified rabbit IgG in 270 μ l of 0.1M Tris-glycine, pH 7.4, 0.15M NaCl, 0.05% sodium azide before the addition of glycerol to 30%. Liquid at -20°C.

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: 0.07-0.4 μ g/ml of this lot detected phosphorylated Akt1/PKB α in lysates from mouse NIH-3T3 fibroblasts treated with 50ng/ml PDGF for 20 minutes.



Immunoblot Analysis

Untreated (Lanes 1 and 3) or PDGF stimulated (lanes 2 and 4) NIH-3T3 cell lysate was resolved by electrophoresis, transferred to nitrocellulose and probed with anti-phospho-Akt1/PKB α (Ser473) (0.4 μ g/ml, lanes 1 and 2). Or Total Anti-Akt1/PKB α (1:1000, Lanes 3 and 4). Proteins were visualized using a goat anti-rabbit secondary antibody conjugated to HRP and a chemiluminescence detection system. Arrow indicates phosphorylated Akt1/PKB α (~60kDa).

General References:

1. Cross, D.A., *et al.*, *Nature* **378**: 785-789, 1995.
2. James, S.R., *et al.*, *Biochem. J.* **315**: 709-713, 1996.
3. Alessi, D.R., *et al.*, *Curr. Biol.* **8**: 69-81, 1998.
4. Alessi, D.R., *et al.*, *Curr. Biol.* **7**: 776-789, 1997.
5. 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 EGTA; 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 3% nonfat dry milk (Catalog # 20-200) in TBS with 0.05% Tween 20 (TBST-MLK) for 1 hour at room temperature with constant agitation.
3. Incubate the nitrocellulose with **0.07-0.4 μ g/ml of anti-phospho-Akt1/PKB α (Ser473)**, diluted in freshly prepared TBST-MLK for overnight with agitation at 4C.
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, 1:3000 dilution was used) in TBST-MLK for 2 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).