

Anti-phospho-Akt1/PKB α (Ser473), clone 11E6

(mouse monoclonal IgG_{1 κ})

Catalog # 05-669

Lot # 22278

Immunogen: KLH conjugated synthetic peptide containing a pSer that corresponds to amino acid residues around phosphoserine 473.

Specificity: Recognizes phosphorylated Akt1/PKB α at Ser473, Mr60kDa. This lot of antibody does not cross-react with Akt1/PKB α dephosphorylated at Ser473.

Species Cross-reactivity: Human and mouse.

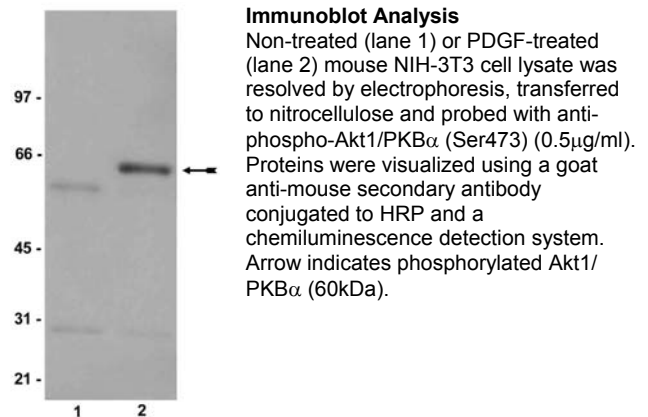
Formulation: 100 μ g of mouse IgG_{1 κ} purified from serum-free cell culture supernatant by subsequent thiophilic adsorption and size exclusion chromatography in 34 μ l 2xPBS containing PEG 3000, sucrose, and 0.09% sodium azide. Frozen at -20°C.

Storage and Stability: Stable for 2 years at -20°C from date of shipment. Thaw aliquots at 37°C. Thawed aliquots may be stored at 4°C up to 3 months. Avoid repeated freeze/thaw cycles. For maximum recovery of product, centrifuge the vial prior to removing the cap.

FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Immunoblot Analysis: 0.5-1 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.



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 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 **0.5-1 μ g/ml of anti-phospho-Akt1/PKB α (S473)** diluted in freshly prepared TBST-MLK for 2 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-mouse HRP conjugated IgG, Catalog # 12-349, 1:3000 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 PBS-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).