

Certificate of Analysis

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

(mouse monoclonal IgG_{1 κ})

Catalog # 05-669

Lot # 0108S0209

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, Mr 60 kDa. This lot of antibody does not cross-react with Akt1/PKB α dephosphorylated at Ser473.

Species Cross-reactivity: Human, mouse, canine and rat.

Formulation: 100 μ g of lyophilized mouse IgG_{1 κ} purified from serum-free cell culture supernatant by subsequent thiophilic adsorption and size exclusion chromatography from 1mL storage buffer (2X PBS, PEG, sucrose, 0.09% sodium azide). Store at -20°C.

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

Handling Recommendations: Reconstitute with 1 mL of H₂O then aliquot into microcentrifuge tubes and store at -20°C. **Avoid repeated freeze/thaw cycles, which may damage IgG and affect product performance.**

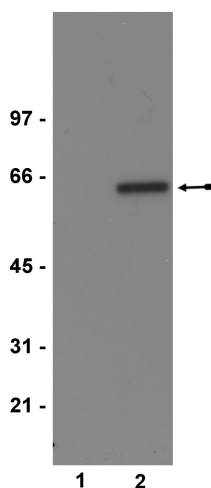
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

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

Additional Research Applications

ELISA: Recommended at 0.05 μ g/mL by an independent laboratory.



Immunoblot Analysis
Representative blot from a previous lot. Non-treated (lane 1) or PDGF-treated (lane 2) 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-mouse secondary antibody conjugated to HRP and a chemiluminescence detection system. Arrow indicates phosphorylated Akt1/PKB α (~60 kDa).

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: 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 1 hour 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 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, 1:5000 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 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).