

## Certificate of Analysis

### Anti-phospho-Akt1/PKB $\alpha$ (Ser473), clone SK703

(rabbit monoclonal IgG)

Catalog # 05-736

Lot # 32515

**Immunogen:** KLH-conjugated, synthetic peptide containing a pSer that corresponds to amino acid position 473 of human Akt1/PKB $\alpha$ . 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 $\alpha$ , 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 $\mu$ g of protein A purified rabbit IgG in 251 $\mu$ l of 70% storage buffer (0.1M Tris-glycine, pH 7.4, 0.15M NaCl, 0.05% sodium azide) and 30% glycerol. Store at -20°C.

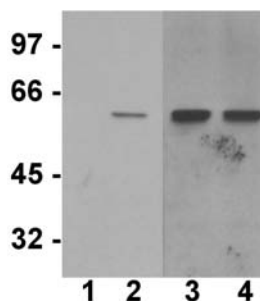
**Storage and Stability:** Stable for 2 years at -20°C from date of shipment.

**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:** 0.05-0.5 $\mu$ g/ml of this lot detected phosphorylated Akt1/PKB $\alpha$  in lysates from mouse NIH-3T3 fibroblasts treated with 100ng/ml PDGF for 20 minutes.



**Immunoblot Analysis**  
Representative blot from a previous lot. 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 $\alpha$  (Ser473) (0.4 $\mu$ g/ml, lanes 1 and 2). Or Total Anti-Akt1/PKB $\alpha$  (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 $\alpha$  (~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 EDTA; 1mM PMSF; 1 $\mu$ g/ml each aprotinin, leupeptin, pepstatin; 1mM Na<sub>3</sub>VO<sub>4</sub>; 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 3% 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 **0.05-0.5 $\mu$ g/ml of anti-phospho-Akt1/PKB $\alpha$  (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, 1:5000 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).