

**Certificate of Analysis**  
**Anti-Myc Tag, clone 4A6, agarose conjugate**  
(mouse monoclonal IgG<sub>1</sub>)  
Catalog # 16-219  
Lot # DAM1437171

**Product Description:** Anti-Myc Tag monoclonal antibody, cross-linked to protein G agarose by dimethylpimelimidate. Immunogen is a KLH-conjugated, synthetic peptide corresponding to amino acids 410-420 (MEQKLISEEDL) of human Myc. Clone 4A6.

**Specificity:** Recognizes and is specific for recombinant proteins containing the Myc epitope tag (EQKLISEEDL) in a variety of sequence contexts. Also recognizes human Myc.

**Species Cross-reactivity:** Human. Other species cross-reactivity not tested.

**Formulation:** 100µg of protein G purified mouse IgG covalently coupled to 100µl of protein G agarose beads and provided as a 50% slurry for a total volume of 200µl in PBS, pH 7.4, containing 0.05% sodium azide. Liquid suspension.

**Storage and Stability:** Stable for 1 year at 4°C from date of shipment. For maximum recovery of product, centrifuge the vial prior to removing the cap.

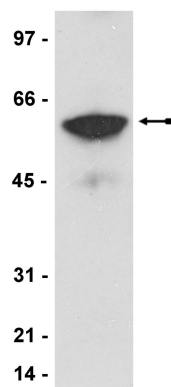
**FOR RESEARCH USE ONLY**  
**NOT FOR USE IN HUMANS**

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### Quality Control Testing

**Immunoprecipitation:** 4µg of anti-Myc Tag, clone 4A6, agarose conjugate immunoprecipitated Myc-tagged Akt/PKB from 250µg of a COS transfected cell lysate, which was then detected by immunoblot analysis, using 0.5µg/ml of anti-Myc Tag, clone 4A6 (Catalog # 05-724).

**Immunoaffinity Purification:** Use to purify Myc-tagged proteins. Elute with 100mM tetraethyl ammonium (TEA), pH 11.5.



#### Immunoprecipitation/Immunoblot Analysis

Representative blot from a previous lot. Myc tagged Akt/PKB was immunoprecipitated from COS transfected lysate using anti-Myc Tag, clone 4A6, agarose conjugate, resolved by electrophoresis, transferred to nitrocellulose and probed with anti-Myc Tag, clone 4A6 (Catalog # 05-724). Proteins were visualized using a goat anti-mouse secondary antibody conjugated to HRP and a chemiluminescence detection system. Arrow indicates Myc-tagged Akt/PKB (~60kDa).

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#### General Reference:

1. Yeong, F. M., *et al.*, *Curr. Biol.* **13**: 2058-64, 2003.

### Immunoprecipitation Protocol

1. Wash the agarose beads with appropriate buffer to remove sodium azide.
2. Dilute the cell lysate before beginning the immunoprecipitation to roughly  $1\mu\text{g}/\mu\text{l}$  total cell protein in a microcentrifuge tube with PBS.
3. Add **4 $\mu\text{g}$  of anti-Myc Tag, clone 4A6, agarose conjugate** to 250-500 $\mu\text{g}$  cell lysate.
4. Gently rock the reaction mixture for 2 hours at 4°C.
5. Collect the agarose beads by pulsing (5 seconds in the microcentrifuge at 14,000 x g), and drain off the supernatant. Wash the beads 3 times with either ice-cold cell lysis buffer or PBS.
6. Resuspend the agarose beads in 60 $\mu\text{l}$  2X Laemmli sample buffer\*\*. The agarose beads can then either be frozen for later use or boiled for 5 minute and collected by a microcentrifuge pulse. Perform SDS-PAGE and immunoblot analysis on a sample of the supernatant.

\*\*Alternatively, proteins may be eluted using 100mM tetraethyl ammonium (TEA), pH 11.5 immediately after Step 5.