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Certificate of Analysis

Anti-Myc Tag, clone 4A6, agarose conjugate

(mouse monoclonal IgG_1)
Catalog # 16-219
Lot # 0611046385

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

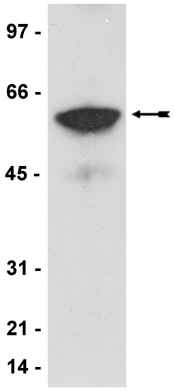
Storage and Stability: Stable for 1 year at 4°C from date of shipment. For maximum recovery of product, centrifuge the vial prior to removing the cap.

FOR RESEARCH USE ONLY
NOT FOR USE IN HUMANS

Quality Control Testing

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

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



Immunoprecipitation/Immunoblot Analysis
Representative blot from a previous lot. Myc tagged Akt/PKB was immuno-precipitated 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 chemi-luminescence detection system. Arrow indicates Myc-tagged Akt/PKB (~60 kDa).

General References:

1. Terpe, K (2003). Overview of tag protein fusions: from molecular and biochemical fundamentals to commercial systems. *Appl Microbiol Biotechnol* **60**: 523-33**.**
2. Yeong, Foong May, *et al* (2003). Identification of a subunit of a novel Kleisin-beta/SMC complex as a potential substrate of protein phosphatase 2A. *Curr Biol* **13**: 2058-64.

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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 µg/µL total cell protein in a microcentrifuge tube with PBS.
3. Add **4 µg of anti-Myc Tag, clone 4A6, agarose conjugate** to 250-500 µg 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 µL 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 100 mM tetraethyl ammonium (TEA), pH 11.5 immediately after Step 5.