

Certificate of Analysis

Anti-Myc Tag, clone 4A6

(mouse monoclonal IgG₁)

Catalog # 05-724

Lot # 0610042765

Immunogen: 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: 200 µg of protein G purified mouse IgG₁ in 200 µL of 0.014 M phosphate buffer, pH 7.6, 0.175 M NaCl, 0.07% sodium azide, and 30% glycerol.

Storage and Stability: Stable for 2 years at -20°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**

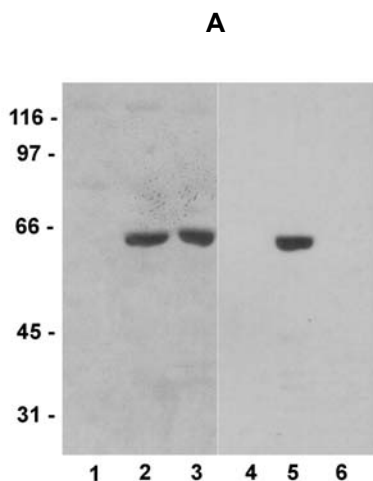
Quality Control Testing

Immunoblot Analysis: 0.5-2 µg/mL of this lot detected Myc-tagged recombinant protein in sequence contexts not well recognized by anti-Myc Tag, clone 9E10 (Catalog # 05-419).

Additional Research Applications

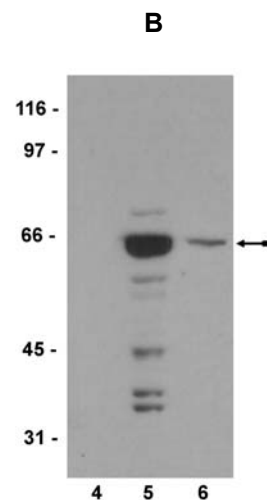
Immunoprecipitation: An independent laboratory has reported that this antibody immunoprecipitates Myc-tagged protein from transfected cells.

Immunocytochemistry: This antibody has been reported by an independent laboratory to detect Myc-tagged nuclear protein in HeLa cells.



Immunoblot Analysis

Panel A. Representative blot from a previous lot. Lysates from NIH/3T3 cells transfected with either empty vector (lanes 1, 4), PP2A A subunit containing the Myc epitope tag sequence MEQKLISEEDLLRKGST (lanes 2, 5), or PP2A A subunit containing the Myc epitope tag sequence MEQKLISEEDLNGST (lanes 3, 6) were resolved by electrophoresis, transferred to nitrocellulose and probed with anti-Myc Tag, clone 4A6 (0.5 µg/mL, lanes 1-3) or anti-Myc Tag, clone 9E10 (1 µg/mL, Catalog # 05-419, lanes 4-6). Proteins were visualized using a goat anti-mouse secondary antibody conjugated to HRP and a chemiluminescence detection system. Arrow indicates Myc-tagged PP2A A subunit (~63kDa).



Panel B. Representative blot from a previous lot. Longer exposure of lanes 4-6 demonstrating weak detection by anti-Myc Tag, clone 9E10 of the Myc-tagged protein containing the epitope tag sequence MEQKLISEEDLNGST

Application References:

1. Yeong, F. M., *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.

Immunoblot Protocol

1. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on a transfected 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 $\mu\text{g}/\text{mL}$ each aprotinin, leupeptin, pepstatin; 1 mM Na_3VO_4 , 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 3% nonfat dry milk (Catalog # 20-200), (TBS-MLK) for 1 hour at room temperature with constant agitation.
3. Incubate the nitrocellulose with **0.5-2 $\mu\text{g}/\text{mL}$ of anti-Myc Tag, clone 4A6**, diluted in freshly prepared TBS-MLK for 2 hours at room temperature with constant agitation.
4. Wash the nitrocellulose twice with water.
5. Incubate the nitrocellulose in the secondary reagent of choice (a goat anti-mouse HRP conjugated IgG, Catalog # 12-349, 1:4000 dilution was used) in TBS-MLK for 30 minutes at room temperature with agitation.
6. Wash the nitrocellulose twice with water.
7. Wash the nitrocellulose in TBS-0.05% Tween 20 for 3-5 minutes.
8. Rinse the nitrocellulose in 4-5 changes of water.
9. Use detection method of choice (enhanced chemiluminescence was used).