

Certificate of Analysis

Anti-Myc Tag, clone 4A6

(mouse monoclonal IgG₁)

Catalog # 05-724MG

Lot # JBC1361197

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: 1mg of protein G purified mouse IgG₁ in 1.0ml of PBS. Frozen at -20°C.

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. Aliquot to avoid repeated thawing and freezing.

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

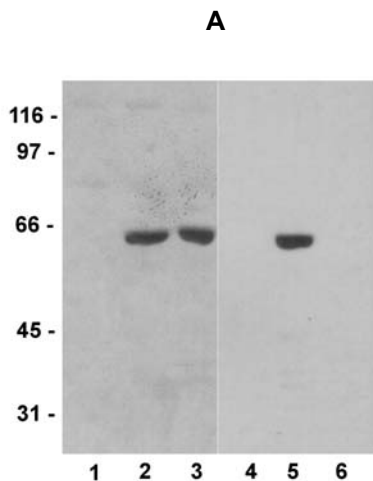
Quality Control Testing

Immunoblot Analysis: 0.5-2µg/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

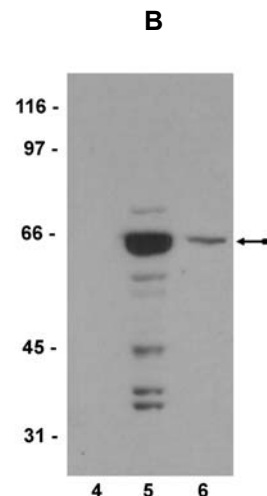
Immunoprecipitation: An independent laboratory has reported that this antibody immunoprecipitates Myc-tagged protein from transfected cells.

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. Lysates from NIH/3T3 cells transfected with either empty vector (lanes 1, 4), PP2A A subunit containing the Myc epitope tag sequence MEQKLISEEDLLRKR GST (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 (0.5µg/ml, lanes 1-3) or anti-Myc Tag, clone 9E10 (1µg/ml, Catalog # 05-419, lanes 4-6). Proteins were visualized using a goat anti-mouse secondary antibody conjugated to HRP and a chemi-luminescence detection system. Arrow indicates Myc-tagged PP2A A subunit (~63kDa).



Panel B. Representative blot. 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

Immunoblot Protocol

1. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on a transfected 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; 1µg/ml each aprotinin, leupeptin, pepstatin; 1mM Na₃VO₄, 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), (TBS-MLK) for 1 hour at room temperature with constant agitation.
3. Incubate the nitrocellulose with **0.5-2µg/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).