

Anti-Myc Tag, clone 4A6

(mouse monoclonal IgG₁)

Catalog # 05-724

Lot # 27433

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: 200µg of protein G purified mouse IgG₁ in 200µl of 0.1M Tris-glycine, pH 7.4, 0.15M NaCl, 0.05% sodium azide before the addition of glycerol to 30%. Liquid 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

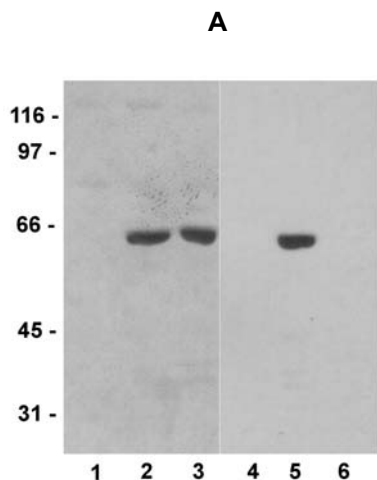
Quality Control Testing

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

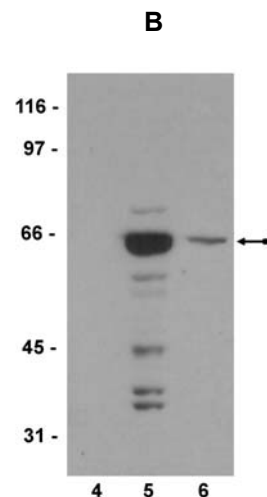
Immunoprecipitation: An independent laboratory has reported that this antibody immunoprecipitates Myc-tagged protein from transfected cells.

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 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 MEQKLISEEDLLRKRGST (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 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

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, 1:2000 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).