



cell signaling solutions

Certificate of Analysis

10 Old Barn Road • Lake Placid, NY 12946
Technical Support: T: 800 548-7853 • F: 518 523-4513
email: techserv@upstate.com
Sales Department: T: 800 233-3991 • F: 781 890-7738
Licensing Dept.: 800 310-4659
www.upstate.com

Anti-Myc Tag, clone 4A6, HRP conjugate

(mouse monoclonal IgG₁)

Catalog # 16-213

Lot # 26862

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: 100µl of protein G purified mouse IgG₁ conjugated to horseradish peroxidase (HRP) in PBS with 0.05% Kathon™ and 0.2mg/ml BSA prior to the addition of glycerol to 50%. Liquid at -20°C.

Storage and Stability: Stable for 1 year 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: A 1:1000-1:5000 dilution of this lot detected Myc-tagged recombinant protein.



Immunoblot Analysis

COS transfected cell lysate was resolved by electrophoresis, transferred to nitrocellulose and probed with anti-Myc Tag, clone 4A6, HRP conjugate (1:5000 dilution). Proteins were visualized using a chemiluminescence detection system. Arrow indicates Myc-tagged protein.

General Reference:

1. Evan, G. I., *et al.* Mol. Cell. Biol. **12**: 3610-3616, 1985.

Immunoblot Protocol

1. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on a lysate containing a Myc-tagged protein (cell lysis buffer: 50mM Tris-HCl, pH 7.4; 1% NP-40; 0.25% sodium deoxycholate; 150mM NaCl; 1mM EDTA; 1mM PMSF; 1 μ g/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 5% nonfat dry milk (Catalog # 20-200), (TBS-MLK) for 60 minutes at room temperature with constant agitation.
3. Incubate the nitrocellulose with a **1:1000-1:5000 dilution of anti-Myc Tag, clone 4A6, HRP conjugate**, diluted in freshly prepared TBS-MLK with agitation for 1.5 hours at room temperature or overnight at 4°C.
4. Wash the nitrocellulose twice with water.
5. Wash the nitrocellulose in TBS-0.05% Tween[®]-20 for 10 minutes.
6. Rinse the nitrocellulose in 4-5 changes of water.
7. Use detection method of choice (enhanced chemiluminescence was used).