

Certificate of Analysis

28820 Single Oak Drive • Temecula, CA 92590
Technical Support: T: 800 437-7500 • F: 800 437-7502
www.millipore.com

Anti-Myc Tag, clone 4A6, biotin conjugate

(mouse monoclonal IgG₁)

Catalog # 16-212

Lot # DAM1479580

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: 100µg of biotin-conjugated protein G purified mouse IgG₁ in 100µl 0.02M Phosphate buffer, 0.25M Sodium Chloride, 0.1% Sodium Azide before the addition of glycerol to 30%. 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: 0.1-1µg/ml 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, biotin conjugate (1µg/ml). Proteins were visualized using a streptavidin-HRP conjugate and a chemiluminescence detection system. Arrow indicates Myc-tagged protein.

General References:

1. Endo, T. A., *et al.* Nature. **387**: 921-921, 1997.
2. Evan, G. I., *et al.* Mol. Cell. Biol. **12**: 3610-3616, 1995.
3. Zugasti, O., *et al.* Mol. Cell. Biol. **21**: 6706-6717, 2001.

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; 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 30 minutes at room temperature with constant agitation.
3. Incubate the nitrocellulose with **0.1-1µg/ml of anti-Myc Tag, clone 4A6, biotin 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. Incubate the nitrocellulose in the Streptavidin-conjugated reagent of choice (a Streptavidin HRP-conjugate, Catalog # 18-152, 0.2µg/ml was used) in TBS-MLK for 1 hour 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).

Unless otherwise stated in our catalog or other company documentation accompanying the product(s), our products are intended for research use only and are not to be used for any other purpose, which includes but is not limited to, unauthorized commercial uses, in vitro diagnostic uses, ex vivo or in vivo therapeutic uses or any type of consumption or application to humans or animals.