

Certificate of Analysis

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Anti-Myc Tag, clone 4A6, biotin conjugate

(mouse monoclonal IgG₁)

Catalog # 16-212

Lot # DAM1408780

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, 1985.
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).

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