

Certificate of Analysis

Anti-Myc Tag, clone 4A6, Alexa Fluor[®] 488 conjugate

(mouse monoclonal IgG₁)

Catalog # 16-224

Lot # 30438

Immunogen: KLH-conjugated, synthetic peptide corresponding to amino acids 410-420 (MEQKLISEEDL) of human Myc. Clone 4A6 conjugated to Alexa Fluor[®] 488.

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.

Applications: Western blotting, immunofluorescence.

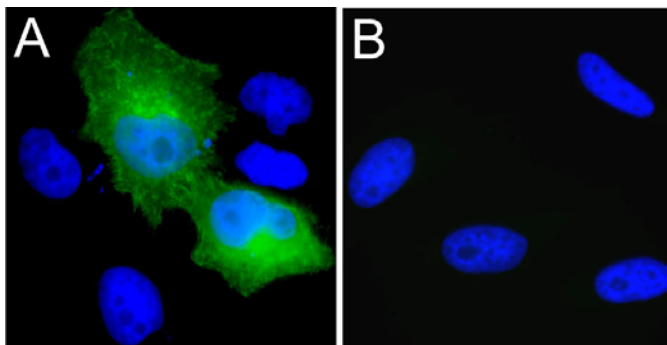
Formulation: 100µg of Alexa Fluor[®] 488 conjugated mouse IgG₁ in 200µl of PBS containing 1% BSA, 0.05% Tween[®]-20, 0.05% sodium azide. Liquid at 4°C.

Storage and Stability: Do Not Freeze. Do not store the material diluted. Stable for 1 year at 4°C from date of shipment. For maximum recovery of product, centrifuge original vial prior to removing cap.

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

Quality Control Testing

Immunocytochemistry: HeLa cells were stained with 1µg/ml of this lot to detect Myc-tagged recombinant protein.

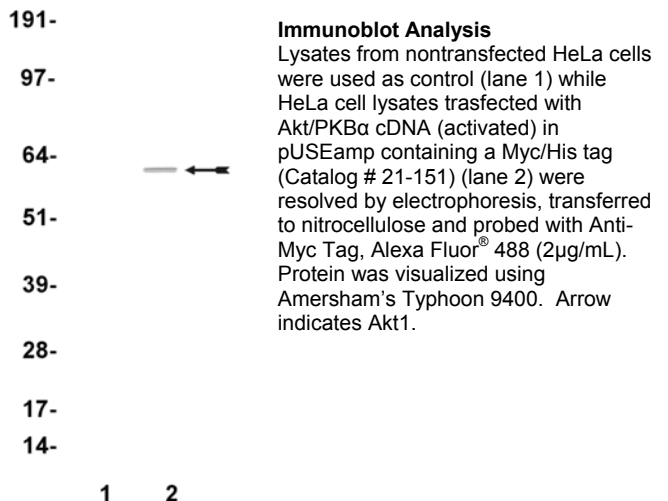


Immunocytochemistry

Panel A: HeLa cells were transfected with Akt/PKBα cDNA (activated) in pUSEamp containing a Myc/His tag (Catalog # 21-151). Cells were stained using Anti-Myc Tag, Alexa Fluor[®] 488 (green) and counterstained with DAPI (blue).

Panel B: HeLa cells were transfected with empty vector pUSEamp (Catalog # 21-147). Cells were stained using Anti-Myc Tag, Alexa Fluor[®] 488 (green) and counterstained with DAPI (blue).

Immunoblot Analysis: RIPA lysates of transiently transfected HeLa cells were resolved by electrophoresis, transferred to nitrocellulose and probed with Anti-Myc Tag, Alexa Fluor[®] 488 (2µg/ml).



Immunoblot Protocol

1. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on a 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.
2. Wet the blotted nitrocellulose in PBS for 5 minutes.
3. Block the blotted nitrocellulose in Odyssey[®] Blocking Buffer (Li-Cor[®], Catalog # 927-40000) for 1 hour at room temperature with constant agitation.
4. Incubate the nitrocellulose with **0.2-2µg/ml of anti-Myc Tag, clone 4A6, Alexa Fluor[®] 488 Conjugate**, diluted in Odyssey[®] Blocking Buffer for 1 hour or longer with agitation at room temperature. Protect from light during incubation.
5. Wash the nitrocellulose 4 times for 5 minutes each at room temperature in PBS-0.05% Tween[®]-20 with agitation. Protect from light.
6. Rinse the nitrocellulose with PBS to remove residual Tween[®]-20. The membrane is now ready to scan.
7. Use detection method of choice (Li-Cor[®] Odyssey[™] Infrared Imaging System or Amersham Biosciences Typhoon Imaging System).

Immunocytochemistry Protocol

1. Plate cells on coverslips in each well of a plate. Place the cells in a CO₂ incubator at 37°C for 24 hours.
2. Remove media and wash the cells with PBS by rinsing 2 times.
3. Add fixative (3.7% formaldehyde) in PBS for 20 minutes at room temperature. Wash two times with PBS for 5 minutes.
4. Permeabilize with 0.5% Triton X-100 for 2 minutes.
5. Wash the cells 2 times with PBS for 5 minutes.
6. Incubate the cells with **1µg/ml of anti-Myc Tag, clone 4A6, Alexa Fluor[®] 488 Conjugate** in PBS for 1 hour.
7. Wash the cells 2 times with PBS for 5 minutes.
8. Mount the coverslip to a slide and dry.
9. Examine the cells under a fluorescent microscope.