

Certificate of Analysis

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Anti-Myc Tag, clone 4A6, Alexa Fluor® 555 conjugate

(mouse monoclonal IgG₁)

Catalog # 16-225

Lot # DAM1416530

Immunogen: KLH-conjugated, synthetic peptide corresponding to amino acids 410-420 (MEQKLISEEDL) of human Myc. Clone 4A6 conjugated to Alexa Fluor® 555.

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.

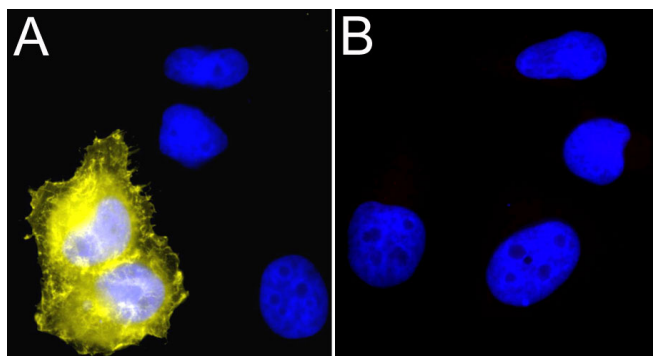
Formulation: 100µg of Alexa Fluor® 555 conjugated mouse IgG₁ in 200µl of PBS containing 1% BSA, and 0.05% sodium azide. Liquid at 4°C.

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.

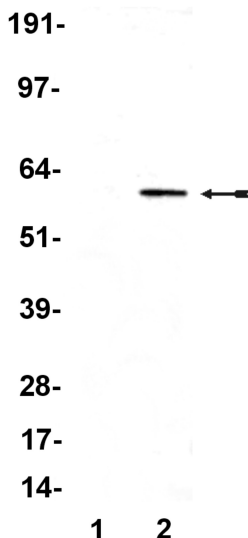


Immunocytochemistry

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® 555 (yellow) 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® 555 (yellow) 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® 555 (2µg/ml).



Immunoblot Analysis

Lysates from nontransfected HeLa cells were used as control (lane 1) while HeLa cell lysates transfected with Akt/PKBα cDNA (activated) in pUSEamp containing a Myc/His tag (Catalog # 21-151) (lane 2) were resolved by electrophoresis, transferred to nitrocellulose and probed with Anti-Myc Tag, Alexa Fluor® 555 (2µg/ml). Protein was visualized using Amersham's Typhoon 9400. Arrow indicates Akt1.

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[®] 555 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[®] 555 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.

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.