

# Anti-Actin, Clone C4

Monoclonal Antibody

Cat. # MAB1501R

Lot # LV1611401

pack size: 100 µg

Store at 2-8°C  
DO NOT FREEZE

FOR RESEARCH USE ONLY  
NOT FOR USE IN HUMANS



## Certificate of Analysis

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Applications	Species Cross-Reactivity	Antibody Isotype	Epitope/Region	Host Species	Molecular Weight	Accession #
WB, IC	H, M, R	IgG2bκ	a.a. 50-70	M	~43 kDa	P68133

### Background

Actin is an abundant cytoskeletal protein found in all cells (True, 1990). The protein's 42 kDa peptide chain assumes two physical forms: globular actin, which serves as a cytoplasmic storage pool, and fibrous actin, which, in conjunction with myosin, generates muscle contraction (True, 1990). In non-muscle cells, actin appears involved in a variety of functions; cell motility, exocytosis, and phagocytosis (True, 1990). Distribution of the six known isoforms of actin - four muscle actins (alpha-skeletal, alpha-vascular smooth, alpha-cardiac, and gamma-enteric smooth) and two cytoplasmic actins (alpha and gamma) is tissue specific (Otey, 1986; Lessard, 1988).

### Presentation

Purified in 0.1M Tris-Glycine (pH 7.4) 150mM NaCl with 0.05% NaN<sub>3</sub>.

### Specificity

MAB1501 is a pan-actin antibody binding to an epitope in a highly conserved region of actin; therefore, it reacts with all six isoforms of vertebrate actin (Lessard, 1988). The epitope recognized by the antibody appears to be located in the N-terminal two thirds of the actin molecule, possibly near amino acids 50-70. It reacts with both globular (G) and filamentous (F) forms of actin and does not interfere with actin polymerization to form filaments at a ratio as high as one antibody per two actin monomers. The antibody however does increase the polymerization when used at a lower ratio of antibody to actin. In addition to labeling myotubes, anti-actin stains myoblasts and fibroblasts (Lessard, 1983). Although clone C4 is prepared as an antibody to chicken gizzard muscles actin, it reacts with actins from all vertebrates, as well as with *Dictyostelium discoideum* and *Physarum polycephalum* actins (Lessard, 1988).

### Concentration

1 mg/mL

### Species Cross-reactivity

Reacts with human, mouse and rat. Other species have not been tested.

### Immunogen

Purified Chicken gizzard actin (Lessard, 1988).

### Molecular Weight

43 kDa

### Method of Purification

Protein G Purified

### Storage and Handling

Maintain at 2-8°C in undiluted aliquots for up to 1 year after date of receipt.

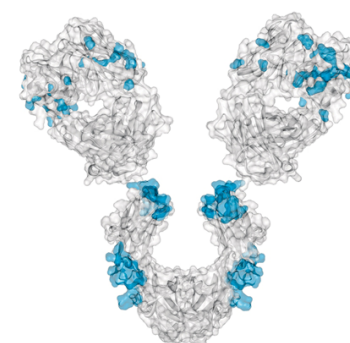
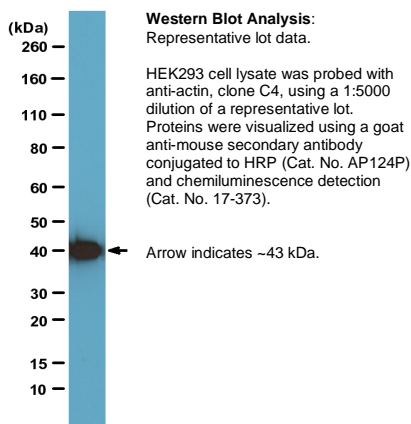
### Control

Western Blot: HEK293 cell lysate

Immunocytochemistry: Hela and NIH/3T3cells

### Quality Control Testing

Evaluated on a representative lot by Western blot on HEK293 lysate using a 1:5000 dilution.



### References

1. Jazirehi, Ali R., et al. (2005). *Cancer Res.* 65: 264-76.
2. Tsou, T., et al. (2004). *Chem. Res. Toxicol.* 17:208-217.
3. Jazirehi, Ali R., et al. (2004). *Cancer Res.* 64: 7117-26.
4. Jazirehi, A.R., et al. (2004). *Mol Cancer Ther.* 3:71-84.
5. Navarro, Claire L., et al. (2004). *Hum. Mol. Genet.* 13:2493-2503.
6. Luciano, L. et al. (2003). *J. Histochem. Cytochem.* 51(2):187-198.
7. True, L. D. (1990) *Atlas of Diag. Immunopat. Gower Medical Pub., N. Y.* 6.5.
8. Lessard, J. (1988). *Cell Motility and the Cytoskeleton.* 10:349-362.
9. Otey, C. et al. (1986). *J. Cell Biol.* 102 :1726-1737.

For additional publications:  
[www.millipore.com/catalogue/item/mab1501R](http://www.millipore.com/catalogue/item/mab1501R)

**APPLICATION LEGEND:** WB Western Blotting IP Immunoprecipitation IC Immunocytochemistry IF Immunofluorescence  
IH Immunohistochemistry (Tissue)

**SPECIES LEGEND:** H Human M Mouse R Rat Rb Rabbit WR Most Common Vertebrates

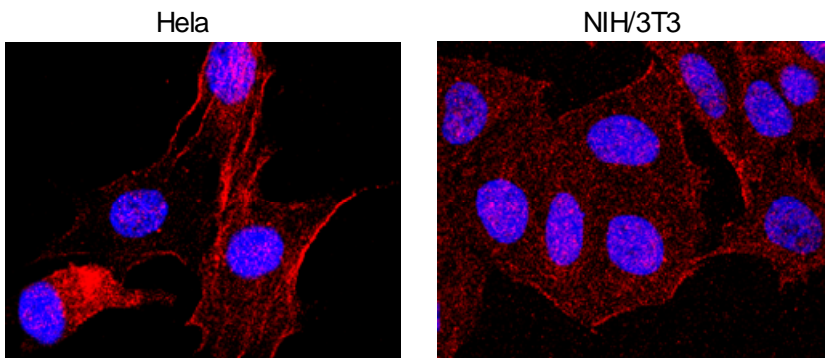
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## Additional Research Applications



### Immunocytochemistry Analysis:

Confocal IF analysis of HeLa and NIH/ cells. Cells were probed with anti-actin, clone C4 (red) using a 1:500 dilution of a representative lot and DAPI (blue) for nuclear staining. Actin antibody was visualized using a donkey anti-mouse IgG conjugated to Cy3 (Cat. No. AP192C). Positive staining pattern reflects both membrane and cytoplasmic staining.

## PROTOCOL

### Western Blot

1. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on a cell lysate or tissue sample (cell lysis buffer: 50 mM Tris-HCl, pH 7.4; 1% NP-40; 0.25% sodium deoxycholate; 150 mM NaCl; 1 mM EDTA; 1 mM PMSF; 1 µg/mL each aprotinin, leupeptin, pepstatin; 1 mM Na<sub>3</sub>VO<sub>4</sub>; 1 mM NaF) and transfer the proteins to PVDF (Immobilon-P). Wash the blotted PVDF twice with Tris/0.05% Tween (TBST).
2. Block the blotted PVDF in freshly prepared TBST - 5% nonfat dry milk (Cat. # 20-200) for 30 minutes at room temp. with constant agitation.
3. Incubate the PVDF with the recommended dilution of anti-actin, clone C4, diluted in freshly prepared TBST - 5% nonfat milk at room temperature for 2 hours (or overnight at 4°C) with agitation.
4. Wash the PVDF 3 times with TBST for 3-5 minutes each.
5. Incubate the PVDF in the secondary reagent of choice (a goat anti-mouse IgG (H+L) HRP conjugated, Catalog # AP124P) in TBST-MILK for 60 minutes at room temperature with agitation.
6. Wash the PVDF 3 times in TBST for 3-5 minutes each wash.
7. Use detection method of choice (enhanced chemiluminescence was used).

### Immunofluorescence

1. Obtain cells at ~ 2 X 10<sup>4</sup> cells/well, grown on an 8-well chamber slide.
2. Aspirate to remove media from cells.
3. Rinse cells 3 times with 1X PBS.
4. Add 200 µl of 4% paraformaldehyde (PFA) to each well.
5. Incubate for 15-20 min at room temperature.
6. Aspirate to remove PFA from the wells. Wash 3 times with ice cold 1X PBS. (4 min each wash). (Cell can be saved in 4°C in 1X PBS).
7. (If it is membrane staining, skip this step) Add 200 µl of permeabilize solution (0.3% Triton X-100/PBS/0.1% BSA) to each well. Incubate for 5 min at room temperature.
8. Aspirate to remove permeabilize solution.
9. Wash 3 times with 1 X PBS, (4 min each wash).
10. Add 200 µl of Blocking Buffer (1XPBS/5% BSA) to each well. Incubate for 30 min at room temperature.
11. Before the end of incubation time, prepare a 1:200 - 1:500 dilution of the antibody in Blocking Buffer.
12. Aspirate to remove the Blocking Buffer.
13. Add the diluted antibody to the designated well. Incubate for 1 hour at room temperature.
14. Aspirate to remove the antibody. Wash 3 times with 1 X PBS. (Each wash-incubate for 4 min at room temperature)
15. Cells are incubated with secondary antibodies (in blocking buffer) at room temperature in dark for 1 hour.
16. Wash 2 times with 1 X PBS. (Each wash, incubate for 4 min at room temperature in dark), Add 200 µl of 0.5 µg/mL of DAPI. Incubate for 5 min at room temperature, in the dark.
17. Aspirate to remove DAPI. Wash 3 times with 1 X PBS. (Each wash-incubate for 5 min at room temperature in dark).
18. Remove plastic chamber and allow slide to dry.
19. Add mounting reagent to slide, place coverslip over slide and seal edges with clear nail polish. Examine under fluorescence microscope.

**Important Note:** During shipment, small volumes of antibody will occasionally become entrapped in the seal of the product vial. For antibodies with volumes of 200 µL or less, we recommend gently tapping the vial on a hard surface or briefly centrifuging the vial on a tabletop centrifuge to dislodge any liquid in the container's cap.

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Lot # LV1611401

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**RELATED PRODUCTS (specific)**

cat #	description
AP124P	■ Goat anti-Mouse IgG, HRP conjugate
AP192C	■ Donkey anti-Mouse IgG, Cy3 conjugate, Species Adsorbed: H, R, B, Eq, Gt, Sh, Rb, Ch, Gp
AB978	■ Anti-Actin
MAB1279	■ Anti-Actin Bundles, clone 420-8
MAB1501X	■ Anti-Actin, a.a. 50-70, Clone C4, AlexaFluor® 488 Conjugated
MAB3128	■ Anti-Actin, clone 10A5
AB3265	■ Anti-Actin, gamma
MAB1501R	■ Anti-Actin, near a.a. 50-70, clone C4
MAB1522	■ Anti-Actin, smooth muscle gamma & alpha actin, clone CGA7
CBL171	■ Anti-Actin, smooth muscle, clone ASM-1
AB3415	■ Anti-F-actin Capping Protein, alpha-1 subunit, aa80-150
IHCR2020-6	■ IHC Select® Anti-Actin, prediluted, clone HHF35
IHCR2002-6	■ IHC Select® Anti-Actin, prediluted, smooth muscle, clone CGA7
FAK100	■ Actin Cytoskeleton / Focal Adhesion Staining Kit

**RELATED PRODUCTS (non-specific)**

cat #	description
IPVH00010	■ Immobilon-P 26.5 cm x 3.75 m Roll PVDF 0.45 µm
IPFL00010	■ Immobilon-FL 26.5 cm x 3.75 m Roll PVDF 0.45 µm
IPVH07850	■ Immobilon-P 7 x 8.4 cm PVDF 0.45 mm (sheet) 50/pk
ISEQ00010	■ Immobilon-P SQ 26.5 cm x 3.75 m 1 roll PVDF 0.2 µm
ISEQ07850	■ Immobilon-P 7 x 8.4 cm PVDF 0.2 mm (sheet) 50/pk
IPFL07810	■ Immobilon-FL 7 x 8.4 cm PVDF 0.45 mm (sheet) 10/pk
WBKLS0100	■ Immobilon Western Chemilum HRP Substrate 100 mL
17-373	■ Spray & Glow™ ECL WB Detection System 1 ea
2060	■ Re-Blot Western Blot Recycling Kit
2500	■ Re-Blot Plus Western Blot Recycling Kit
B2080-175GM	■ Blot Quick Blocker Membrane Blocking Agent 175G

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