

# Anti-Actin, near a.a. 50-70, clone C4



Monoclonal Antibody

Cat. # MAB1501R

Lot # LV1530193

pack size: 100 µg

Store at 2-8°C

FOR RESEARCH USE ONLY  
NOT FOR USE IN HUMANS

## Certificate of Analysis

page 1 of 2

Applications	Species Cross-Reactivity	Antibody Isotype	Epitope/Region	Host Species	Molecular Weight	Accession #
WB, ELISA, IC, IH	A	IgG1k	a.a. 50-70	M	43 kDa	NP_001091

### Background

Actin is an abundant cytoskeletal protein found in all cells (True, 1990). The protein's 42 kD peptide chain assumes two physical forms: globular actin, which may serve as a cytoplasmic storage pool, and fibrous actin, which, in conjunction with myosin, generates muscle contraction (True, 1990). In non-muscle cells, actin appears to be involved in a variety of functions, such as 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 mouse monoclonal IgG1k in buffer containing 0.02 M phosphate buffer, 0.25 M NaCl with 0.1% sodium azide, pH 7.6.

### Concentration

1 mg/mL

### Specificity

MAB1501R is a pan-actin antibody that binds to an epitope in a highly conserved region of actin; therefore, this antibody 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. 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. However, this antibody does increase the extent of 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).

### Species Cross-reactivity

To date, all animal species and cell types with an actin form react by indirect immunofluorescence or immunoblot, including plant actin.

### Immunogen

Purified chicken gizzard actin (Lessard, 1988).

### Molecular Weight

43 kDa

### Method of Purification

Protein A Purified.

### Storage and Handling

Stable for 6 months at 2-8°C from date of receipt.

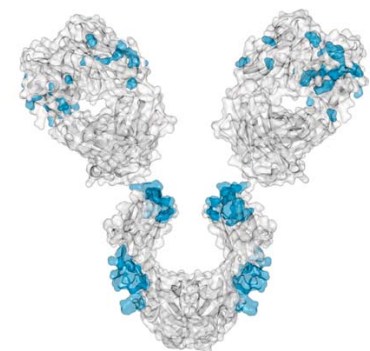
### Control

HeLa whole cell lysate.

### Quality Control Testing

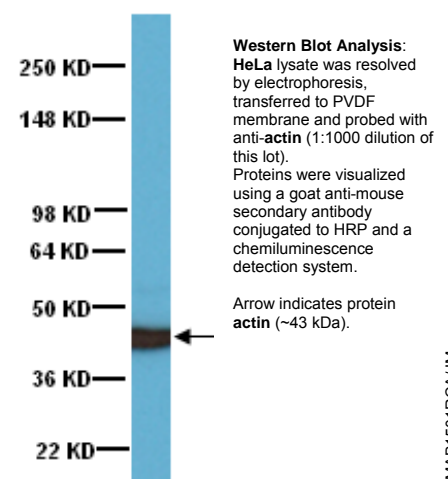
Routinely evaluated by Western Blot on HeLa lysate.

Western Blot Analysis: 1:1000 dilution of this lot detected **actin** on 10 µg of HeLa lysate.



### 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.



**APPLICATION LEGEND:** WB Western Blotting IP Immunoprecipitation IC Immunocytochemistry IF Immunofluorescence IH Immunohistochemistry (Tissue) IH(P) Immunohistochemistry (Paraffin) ELISA Enzyme-linked Immunosorbent Assay

**SPECIES LEGEND:** H Human M Mouse R Rat Rb Rabbit A All Species

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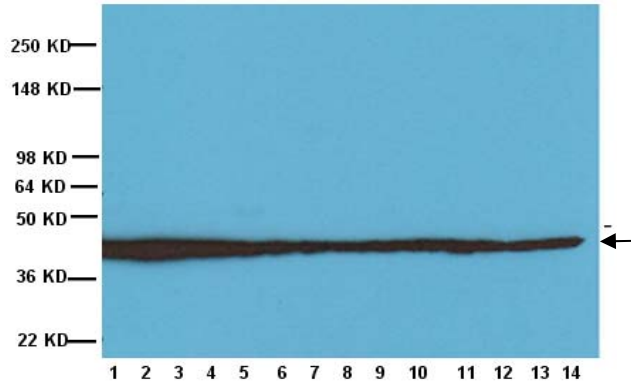
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## Additional Research Applications



### Western Blot Analysis:

Representative image from a previous lot.

A431 (Lane 1), C2C12 (Lane 2), Hek293 (Lane 3), HeLa (Lane 4), HepG2 lysate (Lane 5), Human Placenta lysate (Lane 6), HUVEC (Lane 7), Jurkat (Lane 8), L6 (Lane 9), Mouse Brain (Lane 10), NIH3T3 (Lane 11), PC12 (Lane 12), PC3 (Lane 13), RAW 264.7 (Lane 14) were resolved by electrophoresis, transferred to PVDF membrane and probed with anti-actin (1:1000 dilution of a previous lot). Proteins were visualized using a goat anti-mouse secondary antibody conjugated to HRP and a chemiluminescence detection system.

Arrow indicates protein actin (~43 kDa).

**Immunocytochemistry:** 10 µg/mL dilution from a previous lot was used (methanol fixed mouse 3T3 cells).

**Immunohistochemistry:** 10µg/mL dilution from a previous lot was used for paraffin embedded, 4% formaldehyde, 3% glutaraldehyde, sodium cacodylate treated sections {see Luciano, L et al. 2003}.

**ELISA:** A previous lot was shown to be strongly reactive with the cytoplasmic actin and shows a significant binding to gizzard, skeletal, arterial and cardiac actins. Also shows a significant binding to both Dictyostelium discooidum and Physarum polycephalum.

Optimal working dilutions must be determined by end user.

## PROTOCOL

### Western Blotting

- Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on cell lysate and transfer the proteins to a PVDF membrane. Wash the PVDF membrane twice with water.
- Block the blotted PVDF membrane in freshly prepared 5% BSA with 0.05% Tween®-20 for 1 hour at room temperature with constant agitation.
- Incubate the PVDF with the recommended dilution of anti-Actin, near a.a. 50-70, clone C4 diluted in freshly prepared 5% BSA for 1 hour at room temperature or overnight with agitation at 2-8°C.
- Wash the PVDF 3 times with TBST.
- Incubate the PVDF in the secondary reagent of choice (a donkey anti-mouse HRP conjugate IgG, Catalog # AP192P 1:1000 dilution was used) in 5% milk for 1 hour with agitation at room temperature.
- Wash the PVDF 3-5 times with TBST.
- Use Spray and Glow Catalog # 17-373 to visualize results. Use as directed.

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

### RELATED PRODUCTS (specific)

cat #	description
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
MAB1501	Anti-Actin, clone C4
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
IHC2020-6	IHC Select® Anti-Actin, prediluted, clone HHF35
IHC2002-6	IHC Select® Anti-Actin, prediluted, smooth muscle, clone CGA7

### 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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