Anti-Actin, clone C4

Monoclonal Antibody

Cat. # MAB1501

Lot # LV1519577

pack size: 100 µL

Store at -20ºC

FOR RESEARCH USE ONLY
NOT FOR USE IN HUMANS

Certificate of Analysis

Applications	Species Cross-Reactivity	Antibody Isotype	Epitope/ Region	Host Species	Molecular Weight	Accession #
WB, ELISA, IF	A	IgG1k	Near 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 kDa 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

Mouse monoclonal Ascites fluid, with 0.01% sodium azide.

Specificity

MAB1501 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 vertabrate 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 fillimentous (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).

Affinity Constant

3-15 x 10^8 l/m. (gizzard > cardiac = skeletal muscle actin).

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

Storage and Handling

Maintain at -20°C in undiluted aliquots for up to 12 months after date of receipt. Do not store in a diluted format. Avoid repeated freeze/thaw cycles.

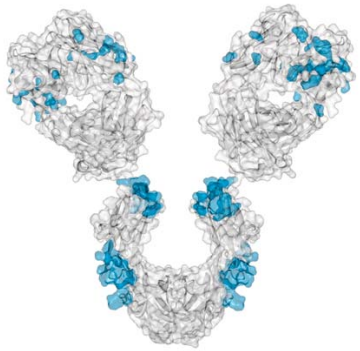
Control

HeLa whole cell lysate.

Quality Control Testing

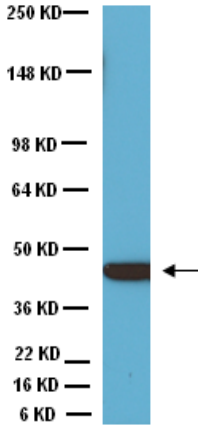
Routinely evaluated by Western Blot on **A431** lysates.

Western Blot Analysis: 1:500 dilution of this lot detected **ACTIN** on 10 ug of **A431** lysates.



References

1. Haan, Claude and Behrmann, Iris, *et al.* (2006). *J Immunol Methods.* 318(1-2):11-9.
2. Calon, F., *et al.* (2005). *Eur J Neurosci.* 22: 617-626.
3. Huang, Chiu-Hui, *et al.* (2007). *J. Biol. Chem.* 282:32442-52.
4. Yang, Ya-Ting, *et al.* (2005). *J. Neurochem.* 93: 513-25.



Western Blot Analysis: **A431** lysate was resolved by electrophoresis, transferred to PVDF membrane and probed with anti-**ACTIN** (1:500 dilution of this 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).

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

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

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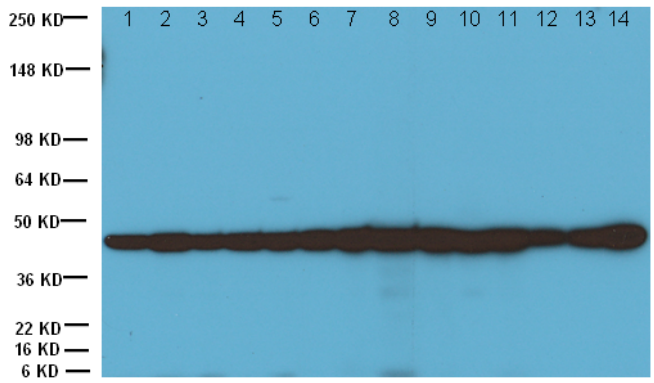
Additional Research Applications



Western Blot Analysis:
Representative image from a previous lot.
A431 (Lane1), C2C12 lysate (Lane2), HEK293 lysate (Lane 3), Hela lysate (Lane 4), HepG2 lysate (Lane 5), Human Placenta lysate (Lane 6), Huvec lysate (Lane 7), Jurkat lysate (Lane 8), L6 lysate (Lane 9), Mouse brain lysate (Lane 10), NIH/3T3 lysate (Lane 11), PC12 lysate (Lane 12), PC3 lysate (Lane 13), RAW264.7 lysate(Lane 14) were resolved by electrophoresis, transferred to PVDF membrane and probed with anti-**ACTIN** (1:500 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).

Indirect immunofluorescence at 1:100: Tissue culture cells -- fix with formaldehyde, treat with methanol or acetone. Glycerinated myofibrils -- fix fibers with formaldehyde, treat with cold methanol. Stains I-bands intensely and stress fibers in human fibroblasts. Cryostat sections (6 µm) -- quick frozen in isopentane, slides treated with gelatin and formaldehyde.

Solid phase binding assay ELISA: 1:800-1:1,000 dilution from a previous lot was shown to be strongly reactive with cytoplasmic actin and shows a significant binding to gizzard, skeletal, arterial and cardiac actins. Also shows a significant binding to both Dictyostelium discoidum and Physarum polycephalum.

Optimal working dilutions must be determined by end user.

PROTOCOL

Western Blotting

1. 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.
2. Block the blotted PVDF membrane in freshly prepared 5% BSA with 0.05% Tween®-20 for 1 hour at room temperature with constant agitation.
3. Incubate the PVDF with the recommended dilution of anti-Actin, Clone C4 diluted in freshly prepared 5% BSA for 1 hour at room temperature or overnight with agitation at 2-8°C.
4. Wash the PVDF 3 times with TBST.
5. 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.
6. Wash the PVDF 3-5 times with TBST.
7. 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
MAB1501R	Anti-Actin, near a.a. 50-70, clone C4
MAB1501X	Anti-Actin, a.a. 50-70, Clone C4, AlexaFluorG10® 488 Conjugated
12-349	Goat Anti-Mouse IgG
AP124P	Goat anti-Mouse IgG, Peroxidase Conjugated, H+L

RELATED PRODUCTS (non-specific)

cat #	description
IPVH00010	Immobilon-P 26.5 cm x 3.75 m Roll PVDF 0.45 um
IPFL00010	Immobilon-FL 26.5 cm x 3.75 m Roll PVDF 0.45 um
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 um
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
WBKLS0050	IMMOBILON WESTERN CHEMILUM HRP SUBSTRATE 50 mL
17-373SP	Spray & Glow™ ECL Western Blotting 40 mL
2060	Re-Blot Western Blot Recycling Kit
2500	Re-Blot Plus Western Blot Recycling Kit
B2080-175GM	Blot Quick Blocker Membrane Blocking Agent 175G
2170	CHEMIBLOCKER-1LT
20-200	IMMUNOBLOT BLOCKING REAGENT 20G

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