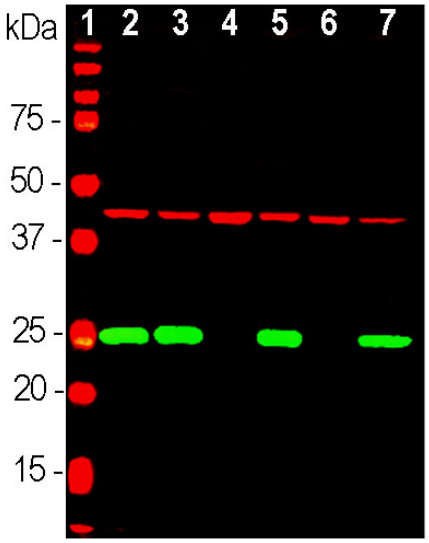
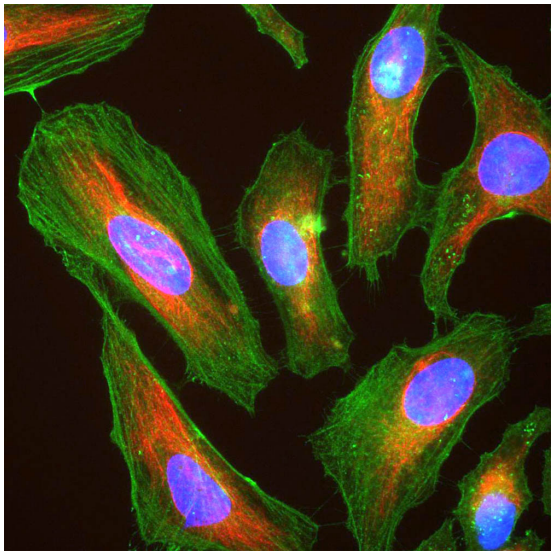
Actin, all Isotypes Mouse Monoclonal Antibody

MCA-5J11

Applications	Host	Isotype	Molecular Wt.	Species Cross-Reactivity
WB, IF/ICC, IHC.	Mouse	IgG1 heavy, κ light	42kDa	Hu, Rt, Ms, Co, Pi, Ho



Western blot analysis of tissue and cell lysates probed with mouse mAb against actin, MCA-5J11, in red. [1] protein standard, [2] rat brain, [3] mouse brain, [4] NIH-3T3, [5] HEK293, [6] HeLa, [7] SH-SY5Y cells. The same blot was simultaneously probed with EnCor chicken pAb to UCHL-1 (CPCA-UCHL1), a marker of neuronal lineage cells, in green.



Immunofluorescent analysis of HeLa cells stained with mAb against actin, MCA-5J11, in green, costained with EnCor chicken pAb against vimentin, CPCA-Vim, in red. The actin antibody labels the submembranous actin-rich cytoskeleton, stress fibers and bundles of actin associated with cell adhesion sites. The vimentin antibody stains a different cytoskeletal network, the intermediate or 10nm filaments. The blue is DAPI staining of nuclear DNA.

Background:

Actin is one of the most abundant and highly conserved proteins of eukaryotes (1-5). Mammalian actins are the product of six distinct but closely related genes with differing distribution patterns in cell types and in tissues. The amino acid sequences of the six human actin gene products are 94-97% identical, so antibodies which bind all six gene products are to be expected. A sequence alignment of the six human actin can be downloaded from here. The molecular weight of all six proteins is ~42kDa, and one or more actins is found in essentially every type of crude cellular and tissue extract. As a result, antibodies to actin are widely used as western blotting standards to verify that the various steps of the western blotting procedure have been performed correctly. In addition, actin is regarded as a "house keeping" protein which is generally not altered in expression as a result of experimental manipulations. Thus, quantification of the actin band on a western blot can be used as a loading control. The actin isotypes were originally classified as α, β and γ since three different actin spots were detected by 2-dimensional SDS-PAGE. Subsequently, the α spot was found to potentially contain three actin gene products, α-skeletal actin, α-vascular smooth muscle actin and α-cardiac muscle actin. The β spot contained a single protein called simply β-actin while the γ spot may contain either γ-1 and/or γ-2 actin, which are enteric and smooth muscle actins respectively. The six mammalian gene products are between 94 and 97% identical, with most of the variability seen at the N-terminus (2). Despite the similarity between the 6 gene products there is some evidence of functional differences between them (6). Actin cycles between monomeric (G-actin) and polymeric microfilaments (F-actin) in a highly regulated manner under the influence of a variety of actin capping and severing proteins the Rho family GTPases, the various Rac, Rho and CDC42 proteins (7).

The MCA-5J11 was made against an actin preparation derived from bovine brain. We have shown that MCA-5J11 binds all six actin gene products, (see supplemental data here), not unexpected given the 94-97% amino acid identity of the 6 proteins. As a result MCA-5J11 will detect all actin proteins present in any mammalian cell or tissue extract, making it a useful and versatile western blotting standard. MCA-5J11 works well for IF, ICC and IHC (see data under "Additional Info" tab).

EnCor Biotechnology Inc.

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HGNC Name: *ACT*
UniProt: *P63267, P60709, P62736, P63261, P68133, P68032*
RRID: *AB_2572218*
Immunogen: *Actin preparation from bovine brain*
Format: *Purified antibody at 1mg/mL in 50% PBS, 50% glycerol plus 5mM NaN_3*
Storage: *Store at 4°C for short term and -20°C for long term*
Recommended dilutions:
WB: 1:1,000. IF/ICC or IHC: 1:500-1,000.

References:

1. Pollard TD and Cooper JA. Actin, a central player in cell shape and movement. Science 326:1208-12 (2009).
2. Vandekerckhove J. and Weber K. At least six different actins are expressed in a higher mammal: an analysis based on the amino acid. sequence of the amino-terminal tryptic peptide. J. Mol. Biol. 126:783–802 (1978).
3. Dominguez R, Holmes KC. Actin structure and function. Annu. Rev. Biophys. 40:169-86 (2011).
4. Davidson AJ, Wood W. Unravelling the Actin Cytoskeleton: A New Competitive Edge? Trends Cell Biol. 26:569-76 (2016).
5. Blanchoin L, et al. Actin dynamics, architecture, and mechanics in cell motility. Physiol. Rev. 94:235-63 (2014).
6. Skruber et al. Reconsidering an active role for G-actin in cytoskeletal regulation. J. Cell Sci. 10:131 (2017).
7. Hall A. Rho GTPases and the actin cytoskeleton. Science 279:509-14 (1998).

This antibody is fairly new so has not been widely used in peer-reviewed publications. However we will add such citations as and when we discover them.

1. Moradi, M. Differential roles of α-, β- and γ-actin isoforms in regulation of cytoskeletal dynamics and stability during axon elongation and collateral branch formation in motoneurons PhD Thesis University of Würzburg 2017.

Abbreviation Key:

mAb—Monoclonal Antibody pAb—Polyclonal Antibody WB—Western Blot IF—Immunofluorescence ICC—Immunocytochemistry IHC—Immunohistochemistry E—ELISA Hu—Human Mo—Monkey Do—Dog Rt—Rat Ms—Mouse Co—Cow Pi—Pig Ho—Horse Ch—Chicken Dr—D. rerio Dm—D. melanogaster Sm—S. mutans Ce—C. elegans Sc—S. cerevisiae Sa—S. aureus Ec—E. coli.

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