

**Ordering Information**  
**Web** www.encorbio.com  
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**HGNC name:** ACTA1, ACTA2, ACTC1, ACTBM, ACTG1, ACTG2  
**RRID:** AB\_2572220

**Host Species:** Mouse

**Immunogen:** Actin preparation derived from bovine brain

**Format:** affinity purified at 1mg/mL in PBS, 50% glycerol, 5mM NaNa<sub>3</sub>

**Storage:** Store at 4°C for short term and at -20°C for long term. Avoid repeated freeze / thaw cycles.

**Recommended dilutions:**

WB: 1:1,000

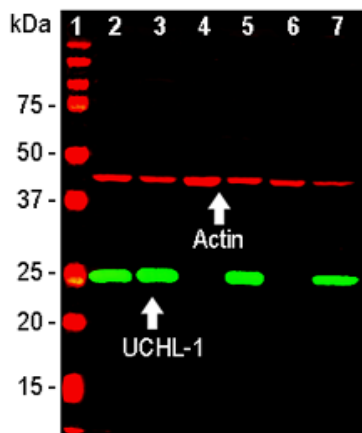
IF/IHC: 1:500-1:1,000

**Application notes:** Western blotting standard; Stains Actin cytoskeleton

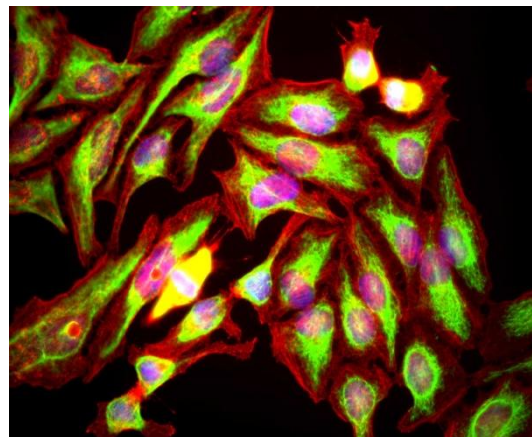
## References:

- Pollard TD1 and Cooper JA. Actin, a central player in cell shape and movement. *Science* 326:1208-12 (2009).
- 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).

Applications	Host	Isotype	Molecular Wt.	Species Cross-Reactivity
WB, ICC/IF, IHC	M	IgG1	42 kDa	H, M, R



**Western blot analysis** of tissue and cell lysates with Mouse mAb against Actin (MCA-5J11, 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 Chicken pAb to UCHL-1 (CPCA-UCHL-1, green).



**Immunofluorescent analysis** of HeLa cells stained with Mouse mAb against Actin (MCA-5J11, red), costained with Chicken pAb against Vimentin (CPCA-Vim, green). The Actin antibody labels submembranous actin-rich cytoskeletons, stress fibers, and bundles of actin associated with cell adhesion sites. The Vimentin antibody stains a different cytoskeletal network, the intermediate filaments. The blue is DAPI staining of nuclear DNA.

**Background:** [Actin](#) is one of the most abundant and highly conserved proteins of eukaryotes (1). Mammalian actins are the product of six different genes with differing distribution patterns in cell types and in tissues. The molecular weight of all six proteins is ~42 kDa, 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 much in expression as a result of experimental manipulations. Thus, quantification of the actin band on the western blot is used as a loading control.

The actin isotypes were originally classified as  $\alpha$ ,  $\beta$  and  $\gamma$  forms based on their charges which resulted in different mobilities in the isoelectric focusing dimension as seen on 2-dimensional SDS-PAGE. Subsequently, the " $\alpha$  spot" was found to potentially contain three actin gene products,  $\alpha$ -skeletal actin,  $\alpha$ -vascular smooth muscle actin, and  $\alpha$ -cardiac muscle actin. The " $\beta$  spot" contained a single protein called simply  $\beta$ -actin, while the " $\gamma$  spot" may contain both  $\gamma$ -1 actin and  $\gamma$ -2, which are enteric and smooth muscle actins respectively.

The six mammalian gene products are very similar as shown by the sequence alignment of the human proteins, which can be downloaded at <http://encorbio.com/Alignments/Actins.pdf>. The most closely related actin isotypes are 97% identical, while the distantly related are 94% identical, with most of the variability at the N-terminus (2). With this level of similarity, antibodies to any one protein may recognize all six isotypes, as is the case with MCA-5J11 (see supplemental data at <http://encorbio.com/Data/5J11.html>), and can work on any mammalian cell type or tissue extract. MCA-5J11 also works in immunocytochemical experiments, strongly labeling filopodia, membrane ruffles and stress fibers of the cells, all known to be rich in actin.

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