Anti-Actin Antibody [JJ09-29]

ET1701-80

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat, Zebrafish
Applications:	WB, IF-Cell, IF-Tissue, IHC-P, IP
Molecular Wt:	Predicted band size: 42 kDa
Clone number:	JJ09-29

Description: All eukaryotic cells express Actin, which often constitutes as much as 50% of total cellular protein. Actin filaments can form both stable and labile structures and are crucial components of microvilli and the contractile apparatus of muscle cells. While lower eukaryotes, such as yeast, have only one Actin gene, higher eukaryotes have several isoforms encoded by a family of genes. At least six types of Actin are present in mammalian tissues and fall into three classes. α-Actin expression is limited to various types of muscle, whereas β-Actin and γ-Actin are the principle constituents of filaments in other tissues. Members of the small GTPase family regulate the organization of the Actin cytoskeleton. Rho controls the assembly of Actin stress fibers and focal adhesion. Rac regulates Actin filament accumulation at the plasma membrane. Cdc42 stimulates formation of filopodia.

Immunogen: Synthetic peptide within Human Actin aa 45-80 / 377.

Positive control: HeLa cell lysate, A431 cell lysate, NIH/3T3 cell lysate, PC-12 cell lysate, mouse brain tissue lysate, mouse colon tissue lysate, rat brain tissue lysate, rat colon tissue lysate, human colon carcinoma tissue, hybrid fish (crucian-carp) brain tissue lysate, hybrid fish (crucian-carp) kidney tissue lysate, NIH/3T3, mouse cardiac muscle tissue, mouse smooth muscle tissue.

Subcellular location: Cytoplasm, Cytoskeleton.

Database links: SwissProt: P68133 Human | P68134 Mouse | P68136 Rat
Entrez Gene: 407658 Zebrafish

Recommended Dilutions:

WB	1:5,000
IF-Cell	1:100-1:500
IF-Tissue	1:100-1:500
IHC-P	1:50-1:200
IP	Use at an assay dependent concentration.

Storage Buffer: 1*TBS (pH7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Store at +4℃ after thawing. Aliquot store at -20℃ or -80℃. Avoid repeated freeze / thaw cycles.

Purity: Protein A affinity purified.

Images

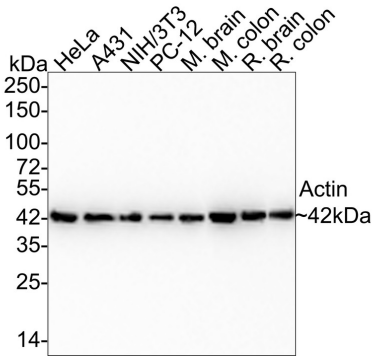


Fig1: Western blot analysis of Actin on different lysates with Rabbit anti-Actin antibody (ET1701-80) at 1/5,000 dilution.

Lane 1: HeLa cell lysate (15 µg/Lane)
Lane 2: A431 cell lysate (15 µg/Lane)
Lane 3: NIH/3T3 cell lysate (15 µg/Lane)
Lane 4: PC-12 cell lysate (15 µg/Lane)
Lane 5: Mouse brain tissue lysate (20 µg/Lane)
Lane 6: Mouse colon tissue lysate (20 µg/Lane)
Lane 7: Rat brain tissue lysate (20 µg/Lane)
Lane 8: Rat colon tissue lysate (20 µg/Lane)

Predicted band size: 42 kDa
Observed band size: 42 kDa

Exposure time: 24 seconds;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (ET1701-80) at 1/5,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:100,000 dilution was used for 1 hour at room temperature.

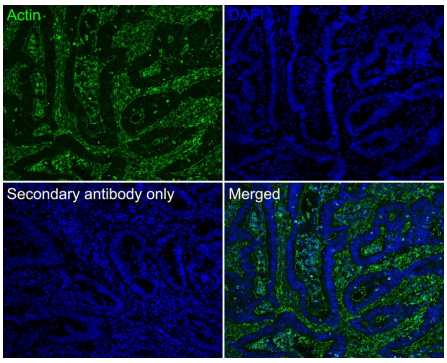


Fig2: Immunofluorescence analysis of paraffin-embedded human colon carcinoma tissue labeling Actin with Rabbit anti-Actin antibody (ET1701-80) at 1/100 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 10% negative goat serum for 1 hour at room temperature, washed with PBS, and then probed with the primary antibody (ET1701-80, green) at 1/100 dilution overnight at 4 ℃, washed with PBS.

Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclei were counterstained with DAPI (blue).

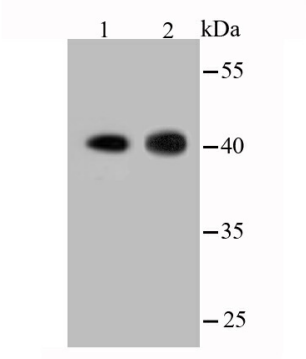


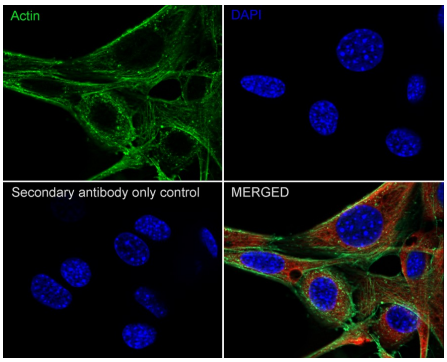
Fig3: Western blot analysis of Actin on different lysates. Proteins were transferred to a PVDF membrane and blocked with 5% BSA in PBS for 1 hour at room temperature. The primary antibody (ET1701-80, 1/500) was used in 5% BSA at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:5,000 dilution was used for 1 hour at room temperature.

Positive control:

Lane 1: Hybrid fish (crucian-carp) brain tissue lysate

Lane 2: Hybrid fish (crucian-carp) kidney tissue lysate

Fig4: Immunocytochemistry analysis of NIH/3T3 cells labeling Actin with Rabbit anti-Actin antibody (ET1701-80) at 1/100 dilution.



Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-Actin antibody (ET1701-80) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

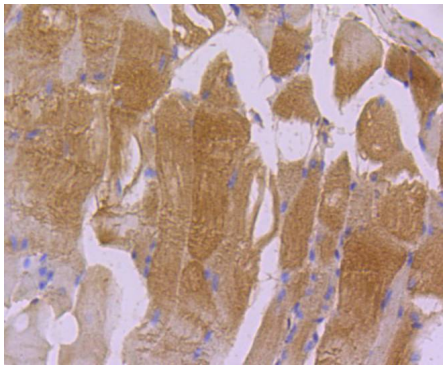


Fig5: Immunohistochemical analysis of paraffin-embedded mouse cardiac muscle tissue using anti-Actin antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1701-80, 1/50) for 30 minutes at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

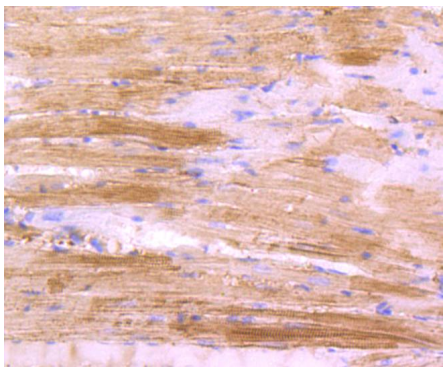


Fig6: Immunohistochemical analysis of paraffin-embedded mouse smooth muscle tissue using anti-Actin antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1701-80, 1/50) for 30 minutes at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

Background References

1. Moilanen AM et al. WDR12, a Member of Nucleolar PeBoW-Complex, Is Up-Regulated in Failing Hearts and Causes Deterioration of Cardiac Function. PLoS One 10:e0124907 (2015).
2. Rafatian N et al. Cardiac macrophages and apoptosis after myocardial infarction: effects of central MR blockade. Am J Physiol Regul Integr Comp Physiol 307:R879-87 (2014).