Anti-Actin Antibody [JF47-01]

ET1702-52

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat, Zebrafish
Applications:	WB, IHC-P, IF-Cell, FC
Molecular Wt:	Predicted band size: 42 kDa
Clone number:	JF47-01

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 303-349 / 377.

Positive control: HeLa cell lysate, C2C12 cell lysate, L6 cell lysate, PC-12 cell lysate, COS-1 cell lysate, Zebrafish tissue lysate, Hybrid fish (crucian-carp) brain tissue lysate, Hybrid fish (crucian-carp) kidney tissue lysate, human skeletal muscle tissue, mouse skeletal muscle tissue, rat skeletal muscle tissue, HeLa, NIH/3T3, L6, PC-12.

Subcellular location: Cytoplasm.

Database links: SwissProt: P68133 Human | P68134 Mouse | P68136 Rat

Recommended Dilutions:

WB	1:5,000-1:20,000
IHC-P	1:200-1:1,000
IF-Cell	1:250-1:500
FC	1:100-1:1,000

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

Storage Instruction: Shipped at 4℃. Store at +4℃ short term (1-2 weeks). It is recommended to aliquot into single-use upon delivery. Store at -20℃ long term.

Purity: Protein A affinity purified.

Images

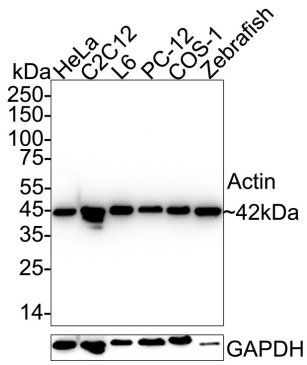

Fig1: Western blot analysis of Actin on different lysates with Rabbit anti-Actin antibody (ET1702-52) at 1/5,000 dilution.

Lane 1: HeLa cell lysate
Lane 2: C2C12 cell lysate
Lane 3: L6 cell lysate
Lane 4: PC-12 cell lysate
Lane 5: COS-1 cell lysate
Lane 6: Zebrafish tissue lysate

Lysates/proteins at 20 µg/Lane.

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

Exposure time: 4 seconds; ECL: K1801;
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 (ET1702-52) at 1/5,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

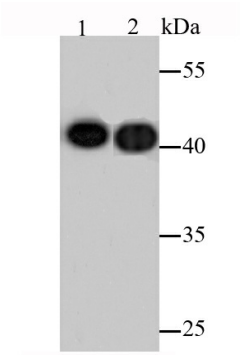

Fig2: Western blot analysis of Actin on different lysates. Proteins were transferred to a PVDF membrane and blocked with 5% BSA in PBS for 1 hour at room temperature. The primary antibody (ET1702-52, 1/500) was used in 5% BSA at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:200,000 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

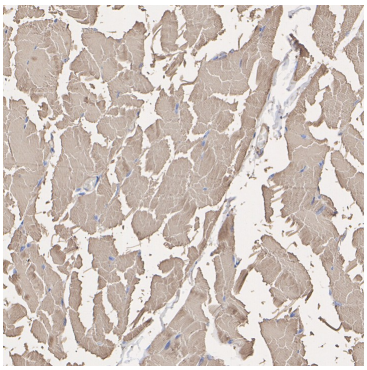

Fig3: Immunohistochemical analysis of paraffin-embedded human skeletal muscle tissue with Rabbit anti-Actin antibody (ET1702-52) at 1/1,000 dilution.

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 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1702-52) at 1/1,000 dilution for 1 hour 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.

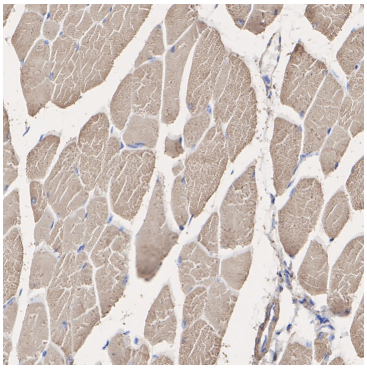

Fig4: Immunohistochemical analysis of paraffin-embedded mouse skeletal muscle tissue with Rabbit anti-Actin antibody (ET1702-52) at 1/1,000 dilution.

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 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1702-52) at 1/1,000 dilution for 1 hour 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.

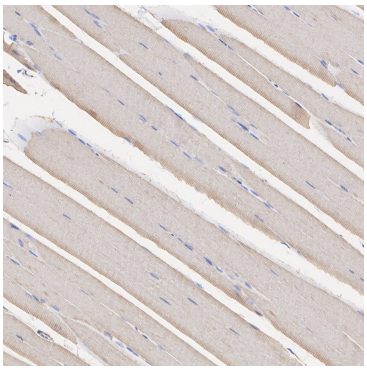

Fig5: Immunohistochemical analysis of paraffin-embedded rat skeletal muscle tissue with Rabbit anti-Actin antibody (ET1702-52) at 1/200 dilution.

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 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1702-52) at 1/200 dilution for 1 hour 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.

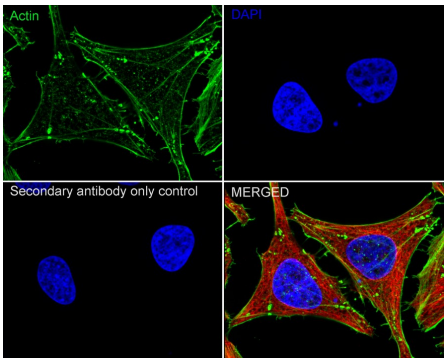

Fig6: Immunocytochemistry analysis of HeLa cells labeling Actin with Rabbit anti-Actin antibody (ET1702-52) at 1/250 dilution.

Cells were fixed in 4% paraformaldehyde for 15 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 15 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 (ET1702-52) at 1/250 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 (HA601187, 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.

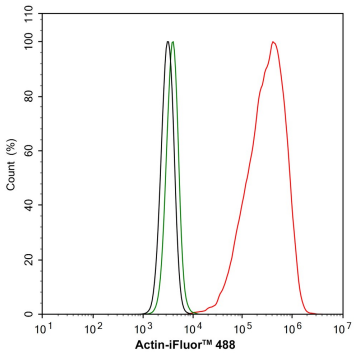

Fig7: Flow cytometric analysis of HeLa cells labeling Actin.

Cells were fixed and permeabilized. Then stained with the primary antibody (ET1702-52, 1/100) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4℃ for an hour, the cells were stained with a iFluor™ 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

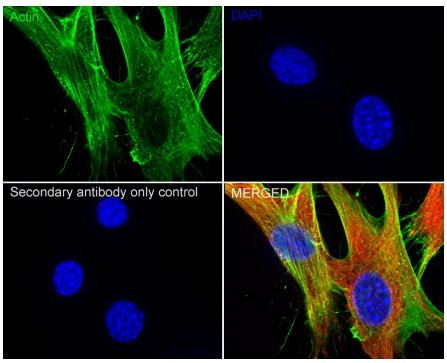

Fig8: Immunocytochemistry analysis of NIH/3T3 cells labeling Actin with Rabbit anti-Actin antibody (ET1702-52) at 1/250 dilution.

Cells were fixed in 4% paraformaldehyde for 15 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 15 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 (ET1702-52) at 1/250 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 (HA601187, 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.

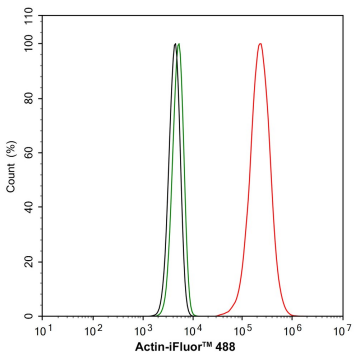

Fig9: Flow cytometric analysis of NIH/3T3 cells labeling Actin.

Cells were fixed and permeabilized. Then stained with the primary antibody (ET1702-52, 1/100) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4℃ for an hour, the cells were stained with a iFluor™ 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

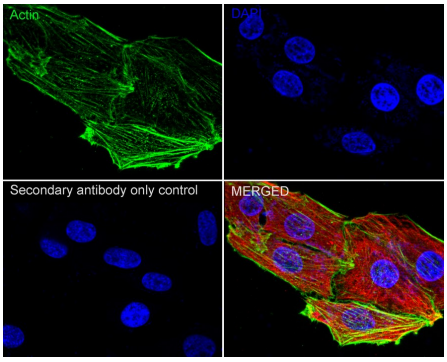

Fig10: Immunocytochemistry analysis of L6 cells labeling Actin with Rabbit anti-Actin antibody (ET1702-52) at 1/500 dilution.

Cells were fixed in 4% paraformaldehyde for 15 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 15 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 (ET1702-52) at 1/500 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 (HA601187, 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.

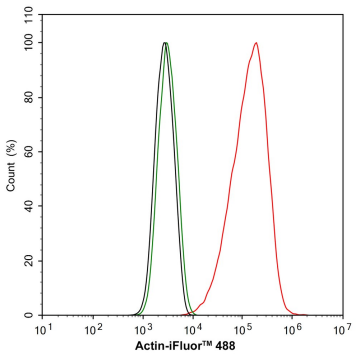

Fig11: Flow cytometric analysis of PC-12 cells labeling Actin.

Cells were fixed and permeabilized. Then stained with the primary antibody (ET1702-52, 1/100) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4℃ for an hour, the cells were stained with a iFluor™ 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

Note: All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE".

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. Tamaki T et al. Therapeutic isolation and expansion of human skeletal muscle-derived stem cells for the use of muscle-nerve-blood vessel reconstitution. Front Physiol 6:165 (2015).