Anti-alpha Actinin 4 Antibody [JU20-23]

ET1706-05

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IP, IHC-P, FC
Molecular Wt:	Predicted band size: 105 kDa
Clone number:	JU20-23

Description: The spectrin gene family encodes a diverse group of cytoskeletal proteins that include spectrins, dystrophins and α-actinins. There are four tissue-specific α-actinins, namely α-actinin-1, α-actinin-2, α-actinin-3 and α-actinin-4, which are localized to muscle and non-muscle cells, including skeletal, cardiac and smooth muscle cells, as well as within the cytoskeleton. Each α-actinin protein contains one Actin-binding domain, two calponin-homology domains, two EF-hand domains and four spectrin repeats, through which they function as bundling proteins that can cross-link F-Actin, thus anchoring Actin to a variety of intracellular structures. Defects in the gene encoding α-actinin-4 are the cause of focal segmental glomerulosclerosis 1 (FSGS1), a common renal lesion characterized by decreasing kidney function and, ultimately, renal failure. are actually sensitive to the Profilin proteins in these foods.

Immunogen: Synthetic peptide within Human alpha Actinin 4 aa 1-50 / 911.

Positive control: A549 cell lysate, HeLa cell lysate, NIH/3T3 cell lysate, Mouse lung tissue lysate, Rat liver tissue lysate, Rat lung tissue lysate, A549, NIH/3T3, mouse liver tissue, rat liver tissue.

Subcellular location: Cell junction. Cytoplasm. Nucleus.

Database links: SwissProt: O43707 Human | P57780 Mouse | Q9QXQ0 Rat

Recommended Dilutions:

WB	1:2,000
IF-Cell	1:100-1:500
IHC-P	1:200
FC	1:1,000
IP	1-2μg/sample

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

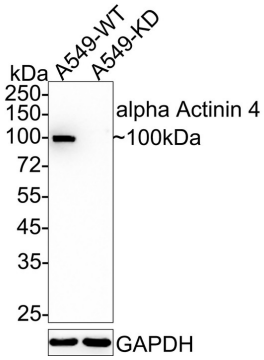

Fig1: Western blot analysis of alpha Actinin 4 on different lysates with Rabbit anti-alpha Actinin 4 antibody (ET1706-05) at 1/2,000 dilution.

Lane 1: A549-si NT cell lysate
Lane 2: A549-si alpha Actinin 4 cell lysate

Lysates/proteins at 10 µg/Lane.

Predicted band size: 105 kDa
Observed band size: 100 kDa

Exposure time: 30 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 (ET1706-05) at 1/2,000 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.

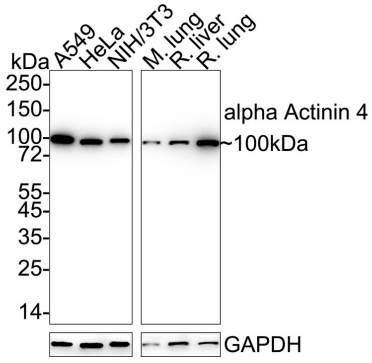

Fig2: Western blot analysis of alpha Actinin 4 on different lysates with Rabbit anti-alpha Actinin 4 antibody (ET1706-05) at 1/2,000 dilution.

Lane 1: A549 cell lysate
Lane 2: HeLa cell lysate
Lane 3: NIH/3T3 cell lysate
Lane 4: Mouse lung tissue lysate
Lane 5: Rat liver tissue lysate
Lane 6: Rat lung tissue lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 105 kDa
Observed band size: 100 kDa

Exposure time: 20 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 (ET1706-05) at 1/2,000 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.

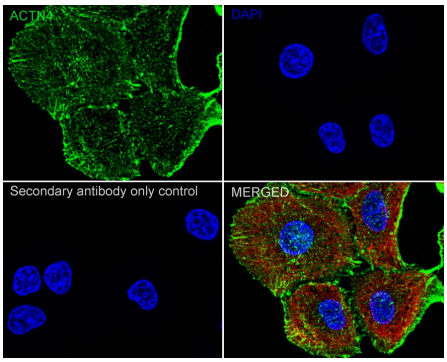

Fig3: Immunocytochemistry analysis of A549 cells labeling alpha Actinin 4 with Rabbit anti-alpha Actinin 4 antibody (ET1706-05) at 1/100 dilution.

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-alpha Actinin 4 antibody (ET1706-05) 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 (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.

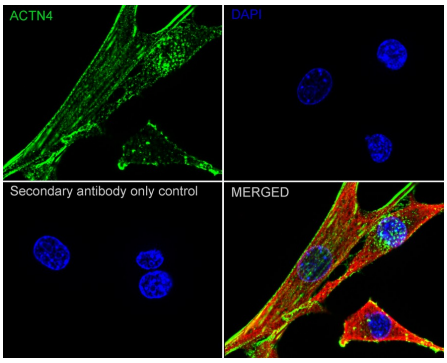

Fig4: Immunocytochemistry analysis of NIH/3T3 cells labeling alpha Actinin 4 with Rabbit anti-alpha Actinin 4 antibody (ET1706-05) at 1/100 dilution.

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-alpha Actinin 4 antibody (ET1706-05) 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 (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.

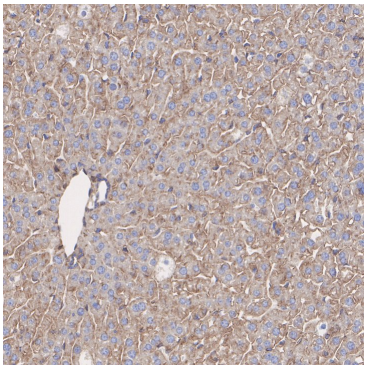

Fig5: Immunohistochemical analysis of paraffin-embedded mouse liver tissue with Rabbit anti-alpha Actinin 4 antibody (ET1706-05) at 1/200 dilution.

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 (ET1706-05) 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.

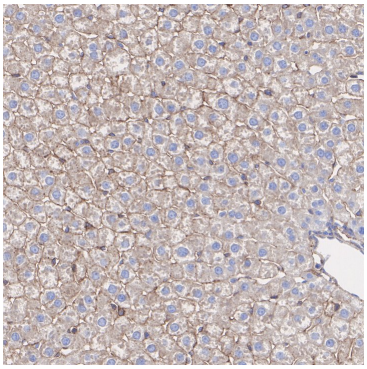

Fig6: Immunohistochemical analysis of paraffin-embedded rat liver tissue with Rabbit anti-alpha Actinin 4 antibody (ET1706-05) at 1/200 dilution.

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 (ET1706-05) 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.

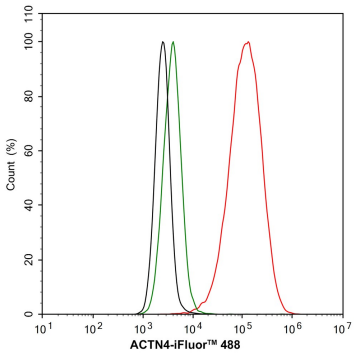

Fig7: Flow cytometric analysis of A549 cells labeling alpha Actinin 4.

Cells were fixed and permeabilized. Then stained with the primary antibody (ET1706-05, 1/1,000) (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).

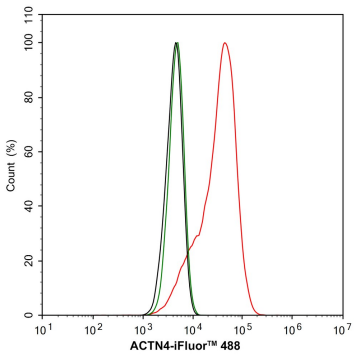

Fig8: Flow cytometric analysis of NIH/3T3 cells labeling alpha Actinin 4.

Cells were fixed and permeabilized. Then stained with the primary antibody (ET1706-05, 1/1,000) (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).

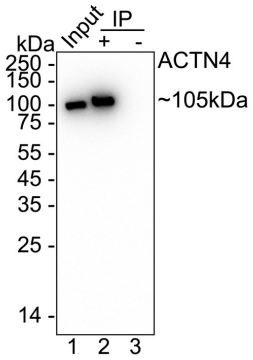

Fig9: alpha Actinin 4 was immunoprecipitated from 0.2 mg A549 cell lysate with ET1706-05 at 2 µg/10 µl beads. Western blot was performed from the immunoprecipitate using ET1706-05 at 1/1,000 dilution. HRP Conjugated Anti-Rabbit IgG for IP Nano-secondary antibody at 1/5,000 dilution was used for 1 hour at room temperature.

Lane 1: A549 cell lysate (input)
Lane 2: ET1706-05 IP in A549 cell lysate
Lane 3: Rabbit IgG instead of ET1706-05 in A549 cell lysate

Blocking/Dilution buffer: 5% NFDM/TBST
Exposure time: 3 seconds; ECL: K1801

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

Background References

1. Piskareva O et al. The development of cisplatin resistance in neuroblastoma is accompanied by epithelial to mesenchymal transition in vitro. Cancer Lett 364(2):142-55 (2015).
2. Lenhart KC et al. GRAF1 deficiency blunts sarcolemmal injury repair and exacerbates cardiac and skeletal muscle pathology in dystrophin-deficient mice. Skelet Muscle 5:27 (2015).