Anti-Filamin A Antibody [SA30-08]

ET1601-3

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat, Monkey
Applications:	WB, IF-Cell, IF-Tissue, IHC-P, FC
Molecular Wt:	Predicted band size: 281 kDa
Clone number:	SA30-08

Description: Actin-binding protein, or filamin, is a 280-kD protein that crosslinks actin filaments into orthogonal networks in cortical cytoplasm and participates in the anchoring of membrane proteins for the actin cytoskeleton. Remodeling of the cytoskeleton is central to the modulation of cell shape and migration. Filamin A, encoded by the FLNA gene, is a widely expressed filamin that regulates the reorganization of the actin cytoskeleton by interacting with integrins, transmembrane receptor complexes, and secondary messengers. At least 31 disease-causing mutations in this gene have been discovered.

Immunogen: Synthetic peptide within Human Filamin A aa 2,598-2,647 / 2,647.

Positive control: HeLa cell lysate, HEK-293 cell lysate, A549 cell lysate, NIH/3T3 cell lysate, C2C12 cell lysate, C6 cell lysate, COS-1 cell lysate, HeLa, C2C12, C6, human uterus tissue, mouse uterus tissue.

Subcellular location: Cytoplasm.

Database links: SwissProt: P21333 Human | Q8BTM8 Mouse
Gene ID: 293860 Rat

Recommended Dilutions:

WB	1:2,000
IF-Cell	1:200-1:500
IF-Tissue	1:50
IHC-P	1:100-1:400
FC	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

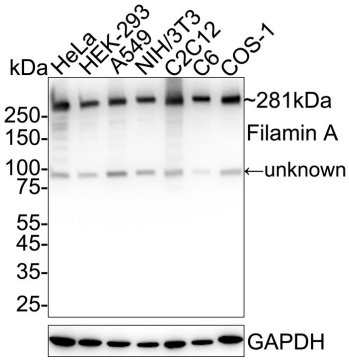

Fig1: Western blot analysis of Filamin A on different lysates with Rabbit anti-Filamin A antibody (ET1601-3) at 1/2,000 dilution.

Lane 1: HeLa cell lysate
Lane 2: HEK-293 cell lysate
Lane 3: A549 cell lysate
Lane 4: NIH/3T3 cell lysate
Lane 5: C2C12 cell lysate
Lane 6: C6 cell lysate
Lane 7: COS-1 cell lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 281 kDa
Observed band size: 281 kDa

Exposure time: 3 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 (ET1601-3) 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.

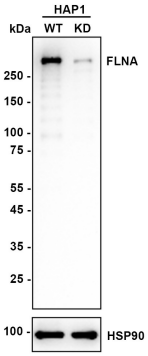

Fig2: Western blot analysis of Filamin A on different lysates with Rabbit anti-Filamin A antibody (ET1601-3) at 1/2,000 dilution.

Lane 1: HAP1-parental cell lysate
Lane 2: HAP1-Filamin A KD cell lysate

Lysates/proteins at 10 µg/Lane.

Predicted band size: 281 kDa
Observed band size: 281 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 (ET1601-3) at 1/2,000 dilution was used in K1803 at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

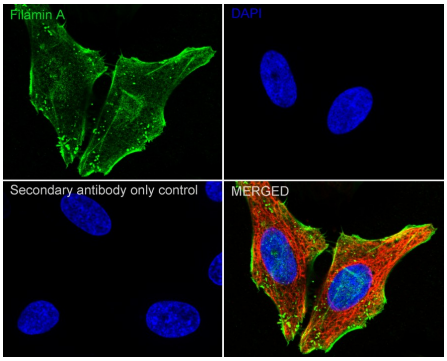

Fig3: Immunocytochemistry analysis of HeLa cells labeling Filamin A with Rabbit anti-Filamin A antibody (ET1601-3) at 1/200 dilution.

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-Filamin A antibody (ET1601-3) at 1/200 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.

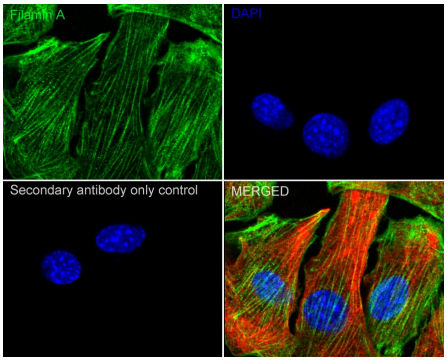

Fig4: Immunocytochemistry analysis of C2C12 cells labeling Filamin A with Rabbit anti-Filamin A antibody (ET1601-3) at 1/500 dilution.

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-Filamin A antibody (ET1601-3) 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.

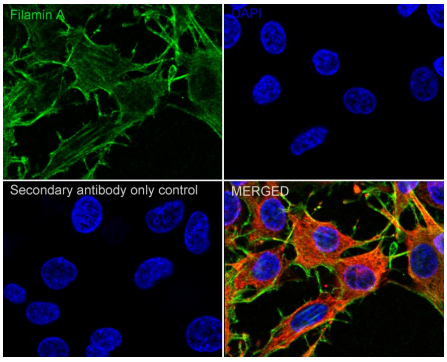

Fig5: Immunocytochemistry analysis of C6 cells labeling Filamin A with Rabbit anti-Filamin A antibody (ET1601-3) at 1/200 dilution.

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-Filamin A antibody (ET1601-3) at 1/200 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.

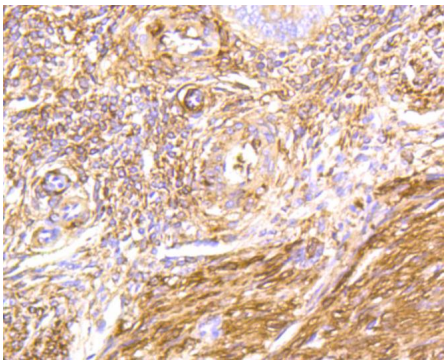

Fig6: Immunohistochemical analysis of paraffin-embedded human uterus tissue using anti-Filamin A antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1601-3, 1/100) 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.

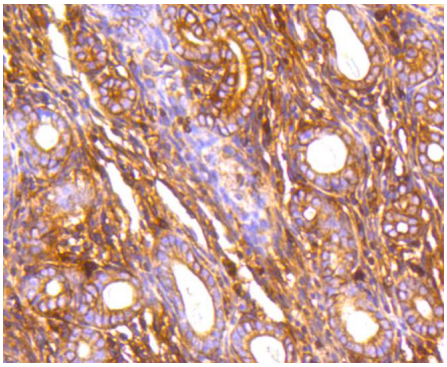

Fig7: Immunohistochemical analysis of paraffin-embedded mouse uterus tissue using anti-Filamin A antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1601-3, 1/400) 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.

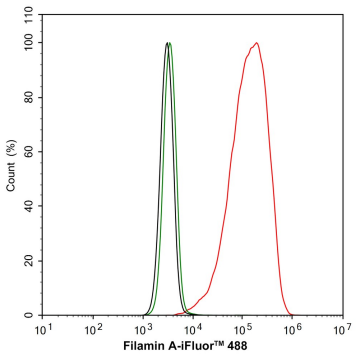

Fig8: Flow cytometric analysis of HeLa cells labeling Filamin A.

Cells were fixed and permeabilized. Then stained with the primary antibody (ET1601-3, 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).

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

Background References

1. Hu J et al. Filamin B regulates chondrocyte proliferation and differentiation through Cdk1 signaling. PLoS One 9:e89352 (2014).
2. Rafizadeh S et al. Functional interaction with filamin A and intracellular Ca2⁺ enhance the surface membrane expression of a small-conductance Ca2+-activated K+ (SK2) channel. Proc Natl Acad Sci U S A 111:9989-94 (2014).