Anti-alpha Actinin Antibody

ER1803-60

Product Type:	Rabbit polyclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat, Monkey
Applications:	WB, IF-Cell, IHC-P, FC
Molecular Wt:	Predicted band size: 103 kDa

Description: Alpha actinins belong to the spectrin gene superfamily which represents a diverse group of cytoskeletal proteins, including the alpha and beta spectrins and dystrophins. Alpha actinin is an actin-binding protein with multiple roles in different cell types. In nonmuscle cells, the cytoskeletal isoform is found along microfilament bundles and adherens-type junctions, where it is involved in binding actin to the membrane. In contrast, skeletal, cardiac, and smooth muscle isoforms are localized to the Z-disc and analogous dense bodies, where they help anchor the myofibrillar actin filaments. This gene encodes a nonmuscle, cytoskeletal, alpha actinin isoform and maps to the same site as the structurally similar erythroid beta spectrin gene. Three transcript variants encoding different isoforms have been found for this gene.

Immunogen: Recombinant protein within Human alpha Actinin aa 388-619 / 892.

Positive control: A431 cell lysate, Rat colon tissue lysate, mouse colon tissue lysates, human breast cancer tissue, rat colon tissue, SH-SY5Y, SiHa.

Subcellular location: Cell junction, Cell membrane, Cell projection, Cytoplasm, Plasma membrane. Cytoskeleton.

Database links: SwissProt: P12814 Human | Q7TPR4 Mouse | Q9Z1P2 Rat

Recommended Dilutions:

WB	1:5,000
IF-Cell	1:200
IHC-P	1:1,000
FC	1:1,000

Storage Buffer: 1*PBS (pH7.4), 0.2% BSA, 50% 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: Immunogen affinity purified.

Images

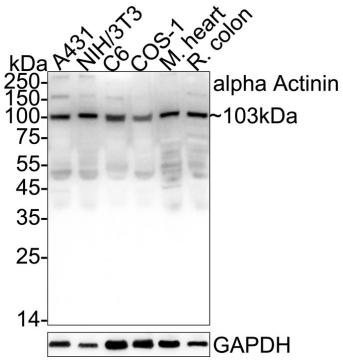

Fig1: Western blot analysis of alpha Actinin on different lysates with Rabbit anti-alpha Actinin antibody (ER1803-60) at 1/5,000 dilution.

Lane 1: A431 cell lysate
Lane 2: NIH/3T3 cell lysate
Lane 3: C6 cell lysate
Lane 4: COS-1 cell lysate
Lane 5: Mouse heart tissue lysate
Lane 6: Rat colon tissue lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 103 kDa
Observed band size: 103 kDa

Exposure time: 10 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 (ER1803-60) 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.

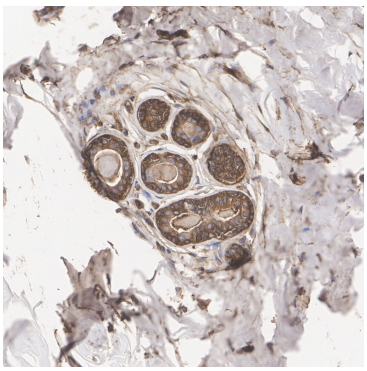

Fig2: Immunohistochemical analysis of paraffin-embedded human breast cancer tissue with Rabbit anti-alpha Actinin antibody (ER1803-60) at 1/1,000 dilution.

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 (ER1803-60) 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.

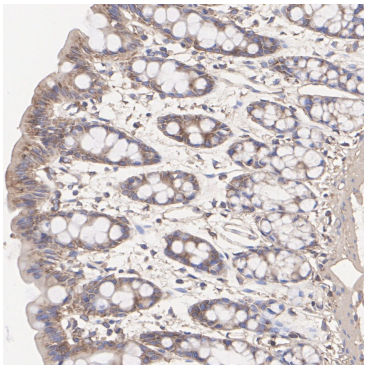

Fig3: Immunohistochemical analysis of paraffin-embedded rat colon tissue with Rabbit anti-alpha Actinin antibody (ER1803-60) at 1/1,000 dilution.

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 (ER1803-60) 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.

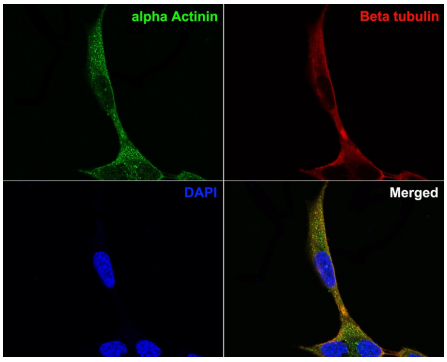

Fig4: Immunocytochemistry analysis of SH-SY5Y cells labeling alpha Actinin with Rabbit anti-alpha Actinin antibody (ER1803-60) at 1/200 dilution.

Cells were fixed in 4% paraformaldehyde for 10 minutes at 37 ℃, permeabilized with 0.05% Triton X-100 in PBS for 20 minutes, and then blocked with 2% negative goat serum for 30 minutes at room temperature. Cells were then incubated with Rabbit anti-alpha Actinin antibody (ER1803-60) at 1/200 dilution in 2% negative goat serum overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. 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™ 647, HA1127) were used as the secondary antibody at 1/1,000 dilution.

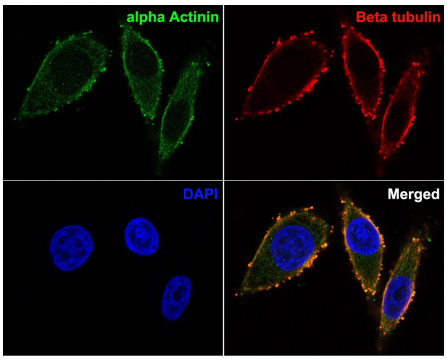

Fig5: Immunocytochemistry analysis of Siha cells labeling alpha Actinin with Rabbit anti-alpha Actinin antibody (ER1803-60) at 1/200 dilution.

Cells were fixed in 4% paraformaldehyde for 10 minutes at 37 ℃, permeabilized with 0.05% Triton X-100 in PBS for 20 minutes, and then blocked with 2% negative goat serum for 30 minutes at room temperature. Cells were then incubated with Rabbit anti-alpha Actinin antibody (ER1803-60) at 1/200 dilution in 2% negative goat serum overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. 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™ 647, HA1127) were used as the secondary antibody at 1/1,000 dilution.

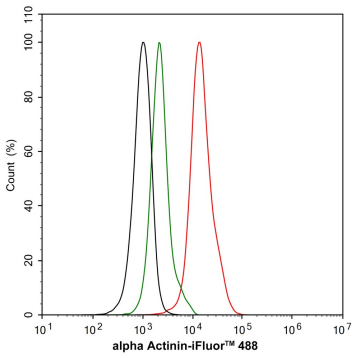

Fig6: Flow cytometric analysis of SH-SY5Y cells labeling alpha Actinin.

Cells were fixed and permeabilized. Then stained with the primary antibody (ER1803-60, 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. Kunishima S et al. ACTN1 mutations cause congenital macrothrombocytopenia. Am J Hum Genet 92:431-438(2013).
2. Izaguirre G et al. The cytoskeletal/non-muscle isoform of alpha-actinin is phosphorylated on its actin-binding domain by the focal adhesion kinase. J Biol Chem 276:28676-28685(2001).