Anti-Calponin Antibody [SI67-01]

ET1606-17

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IF-Tissue, IHC-P
Molecular Wt:	Predicted band size: 33 kDa
Clone number:	SI67-01

Description: Calponin regulates smooth muscle cell contraction and is a marker of smooth muscle cell differentiation. Calponin, an Actin- and Tropomyosin-binding protein, is characterized as an inhibitory factor of smooth-muscle actomyosin activity. Calponin is implicated in the regulation of smooth muscle contraction through its interaction with F-Actin and inhibition of the Actin-activated MgATPase activity of phosphorylated Myosin. Both properties are lost following phosphorylation (primarily at Serine 175) by protein kinase C or calmodulin-dependent protein kinase II. The three forms of Calponin, Calponin 1 (basic Calponin), Calponin 2 (neutral Calponin) and Calponin 3 (acidic Calponin), are found in smooth muscle tissue. Additionally, Calponin 2 is found in heart muscle tissue and Calponin 3 is found in the brain.

Immunogen: Synthetic peptide within Human Calponin aa 248-297 / 297.

Positive control: Mouse stomach tissue lysate, Rat stomach tissue lysate, Hela cell lysate, NIH/3T3 cell lysate, HepG2, mouse colon tissue, rat colon tissue, rat stomach tissue.

Subcellular location: Cytoskeleton,focal adhesion.

Database links: SwissProt: P51911 Human | Q08091 Mouse | Q08290 Rat

Recommended Dilutions:

WB	1:500-1:2,000
IF-Cell	1:50-1:200
IF-Tissue	1:50-1:200
IHC-P	1:5,000

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

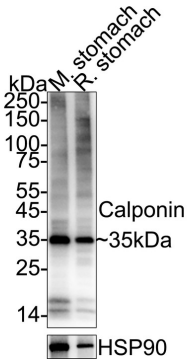

Fig1: Western blot analysis of Calponin on different lysates with Rabbit anti-Calponin antibody (ET1606-17) at 1/1,000 dilution.

Lane 1: Mouse stomach tissue lysate
Lane 2: Rat stomach tissue lysate

Lysates/proteins at 40 µg/Lane.

Predicted band size: 33 kDa
Observed band size: 35 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 (ET1606-17) at 1/1,000 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.

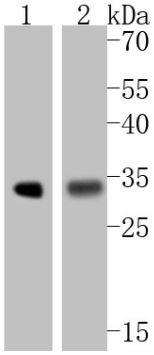

Fig2: Western blot analysis of Calponin on different lysates. Proteins were transferred to a PVDF membrane and blocked with 5% BSA in PBS for 1 hour at room temperature. The primary antibody (ET1606-17, 1/500) was used in 5% BSA at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:5,000 dilution was used for 1 hour at room temperature.
Positive control:
Lane 1: Hela cell lysate
Lane 2: NIH/3T3 cell lysate

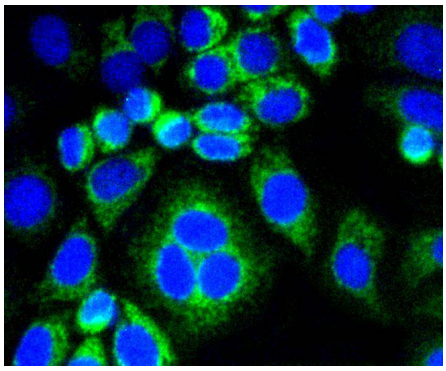

Fig3: ICC staining of Calponin in HepG2 cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1606-17, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).

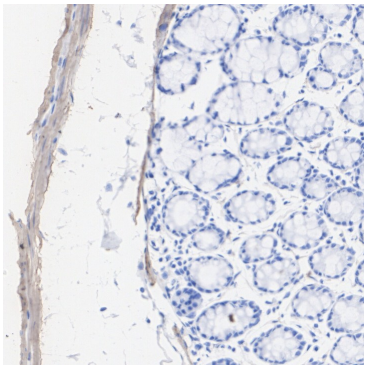

Fig4: Immunohistochemical analysis of paraffin-embedded mouse colon tissue with Rabbit anti-Calponin antibody (ET1606-17) at 1/5,000 dilution.

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 (ET1606-17) at 1/5,000 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.

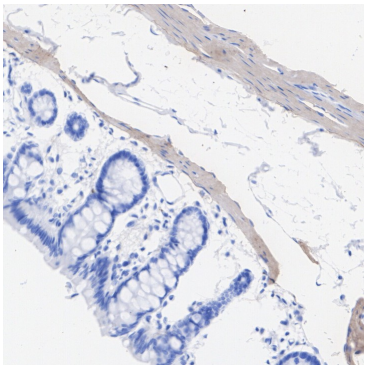

Fig5: Immunohistochemical analysis of paraffin-embedded rat colon tissue with Rabbit anti-Calponin antibody (ET1606-17) at 1/5,000 dilution.

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 (ET1606-17) at 1/5,000 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.

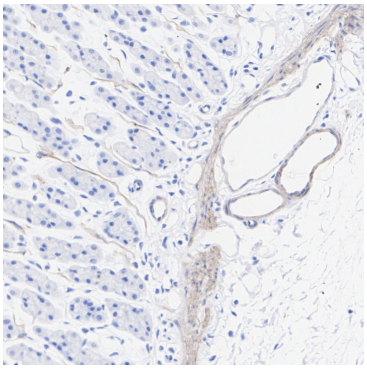

Fig6: Immunohistochemical analysis of paraffin-embedded rat stomach tissue with Rabbit anti-Calponin antibody (ET1606-17) at 1/5,000 dilution.

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 (ET1606-17) at 1/5,000 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.

Background References

1. Battiston KG et al. Monocyte/macrophage cytokine activity regulates vascular smooth muscle cell function within a degradable polyurethane scaffold. Acta Biomater 10:1146-55 (2014).
2. Nyp MF et al. TRIP-1 via AKT modulation drives lung fibroblast/myofibroblast trans-differentiation. Respir Res 15:19 (2014).