Anti-alpha smooth muscle Actin Antibody [SY25-03]

ET1607-43

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IHC-P, FC, IP
Molecular Wt:	Predicted band size: 42 kDa
Clone number:	SY25-03

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 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 N-terminal human alpha smooth muscle Actin.

Positive control: HeLa cell lysate, A431 cell lysate, A549 cell lysate, NIH/3T3 cell lysate, C2C12 cell lysate, L6 cell lysate, mouse colon tissue lysate, rat colon tissue lysate, human tonsil tissue, human lung tissue, human liver tissue, mouse skin tissue, mouse small intestine tissue, Hela.

Subcellular location: Cytoplasm.

Database links: SwissProt: P62736 Human | P62737 Mouse | P62738 Rat

Recommended Dilutions:

WB	1:2,000-1:5,000
IHC-P	1:50-1:200
FC	1:50-1:100
IP	Use at an assay dependent concentration.

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

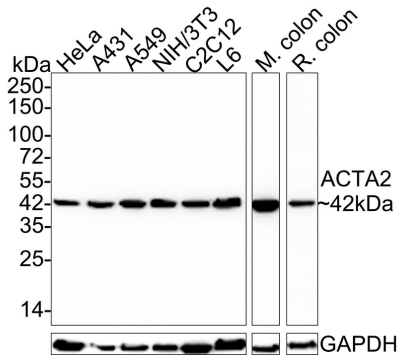

Fig1: Western blot analysis of alpha smooth muscle Actin on different lysates with Rabbit anti-alpha smooth muscle Actin antibody (ET1607-43) at 1/2,000 dilution.

Lane 1: HeLa cell lysate
Lane 2: A431 cell lysate
Lane 3: A549 cell lysate
Lane 4: NIH/3T3 cell lysate
Lane 5: C2C12 cell lysate
Lane 6: L6 cell lysate
Lane 7: Mouse colon tissue lysate
Lane 8: Rat colon tissue lysate

Lysates/proteins at 20 µg/Lane.

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

Exposure time: 1 minute;

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 (ET1607-43) at 1/2,000 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:100,000 dilution was used for 1 hour at room temperature.

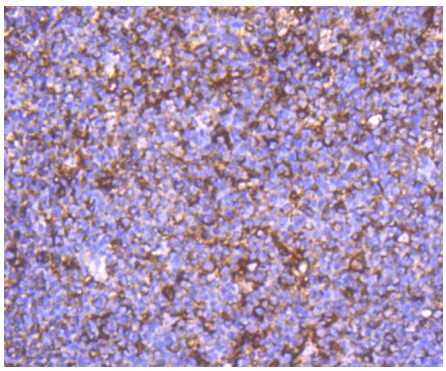

Fig2: Immunohistochemical analysis of paraffin-embedded human tonsil tissue using anti-alpha smooth muscle Actin antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1607-43, 1/50) 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.

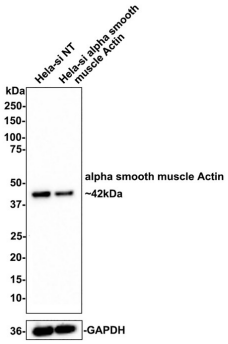

Fig3: Western blot analysis of alpha smooth muscle Actin on different lysates with Rabbit anti-alpha smooth muscle Actin antibody (ET1607-43) at 1/1,000 dilution.

Lane 1: Hela-si NT cell lysate
Lane 2: Hela-si alpha smooth muscle Actin cell lysate

Lysates/proteins at 10 µg/Lane.

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

Exposure time: 2 minutes;
4-20% SDS-PAGE gel.

ET1607-43 was shown to specifically react with alpha smooth muscle Actin in Hela-si NT cells. Weakened band was observed when Hela-si alpha smooth muscle Actin sample was tested. Hela-si NT and Hela-si alpha smooth muscle Actin samples were subjected to SDS-PAGE. Proteins were transferred to a PVDF membrane and blocked with 5% NFDM in TBST for 1 hour at room temperature. The primary antibody (ET1607-43, 1/1,000) and Loading control antibody (Rabbit anti-GAPDH, ET1601-4, 1/10,000) were used in 5% BSA at room temperature for 2 hours. Goat Anti-rabbit IgG-HRP Secondary Antibody (HA1001) at 1:100,000 dilution was used for 1 hour at room temperature.

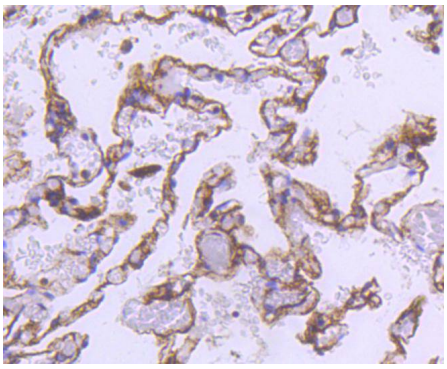

Fig4: Immunohistochemical analysis of paraffin-embedded human lung tissue using anti-alpha smooth muscle Actin antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1607-43, 1/50) 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.

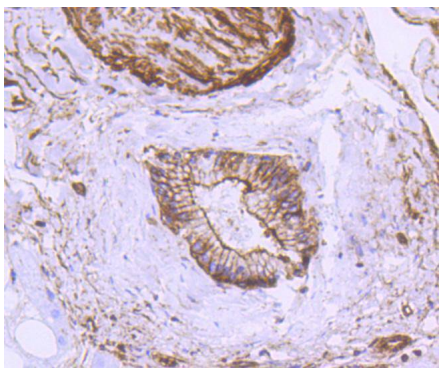

Fig5: Immunohistochemical analysis of paraffin-embedded human liver tissue using anti-alpha smooth muscle Actin antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1607-43, 1/50) 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.

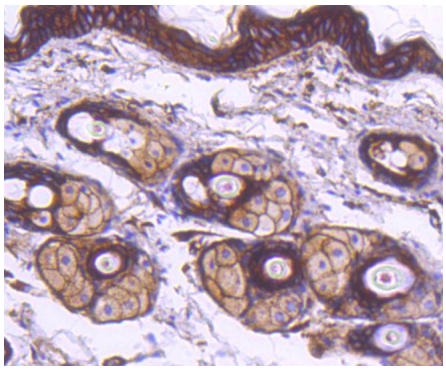

Fig6: Immunohistochemical analysis of paraffin-embedded mouse skin tissue using anti-alpha smooth muscle Actin antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1607-43, 1/50) 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.

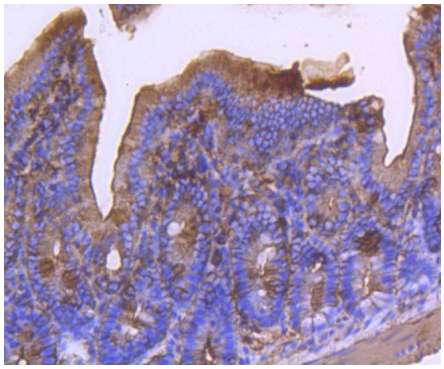

Fig7: Immunohistochemical analysis of paraffin-embedded mouse small intestine tissue using anti-alpha smooth muscle Actin antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1607-43, 1/50) 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.

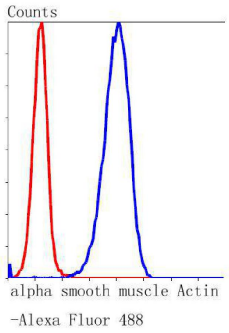

Fig8: Flow cytometric analysis of alpha smooth muscle Actin was done on Hela cells. The cells were fixed, permeabilized and stained with the primary antibody (ET1607-43, 1/50) (blue). After incubation of the primary antibody at room temperature for an hour, the cells were stained with a Alexa Fluor 488-conjugated Goat anti-Rabbit IgG Secondary antibody at 1/1000 dilution for 30 minutes.Unlabelled sample was used as a control (cells without incubation with primary antibody; red).

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

Background References

1. Manetti M et al. Telocytes are reduced during fibrotic remodelling of the colonic wall in ulcerative colitis. J Cell Mol Med 19:62-73 (2015).
2. Ikenaga N et al. A new Mdr2(-/-) mouse model of sclerosing cholangitis with rapid fibrosis progression, early-onset portal hypertension, and liver cancer. Am J Pathol 185:325-34 (2015).