alpha smooth muscle Actin Recombinant Antibody [SY02-64] - Mouse IgG1 (Chimeric) - BSA and Azide free

HA610362

Product Type:	Recombinant Chimeric Antibody, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IHC-P, IF-Cell, FC
Molecular Wt:	Predicted band size: 42 kDa
Clone number:	SY02-64

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-Actin 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: Saos-2 cell lysate, A431 cell lysate, NIH/3T3 cell lysate, C2C12 cell lysate, PC-12 cell lysate, Mouse skin tissue lysate, Rat skin tissue lysate, human small intestine tissue, mouse small intestine tissue, rat small intestine tissue, NIH/3T3, Saos-2.

Subcellular location: Cytoplasm.

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

Recommended Dilutions:

WB	1:5,000
IHC-P	1:80,000
IF-Cell	1:2,000
FC	1:1,000

Storage Buffer: PBS (pH7.4).

Storage Instruction: Store at +4℃ after thawing. Aliquot store at -20℃. Avoid repeated freeze / thaw cycles.

Purity: Protein A affinity purified.

Images

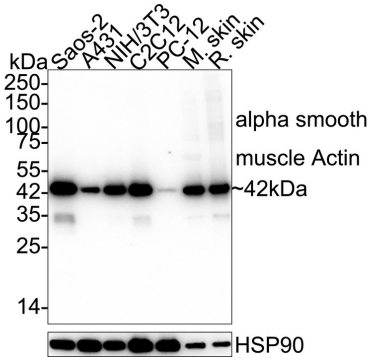

Fig1: Western blot analysis of alpha smooth muscle Actin on different lysates with Mouse anti-alpha smooth muscle Actin antibody (HA610362) at 1/5,000 dilution.

Lane 1: Saos-2 cell lysate
Lane 2: A431 cell lysate
Lane 3: NIH/3T3 cell lysate
Lane 4: C2C12 cell lysate
Lane 5: PC-12 cell lysate
Lane 6: Mouse skin tissue lysate
Lane 7: Rat skin tissue lysate

Lysates/proteins at 20 µg/Lane.

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

Exposure time: 9 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 (HA610362) at 1/5,000 dilution was used in primary antibody dilution (K1803) at 4℃ overnight. Goat Anti-Mouse IgG - HRP Secondary Antibody (HA1006) at 1/50,000 dilution was used for 1 hour at room temperature.

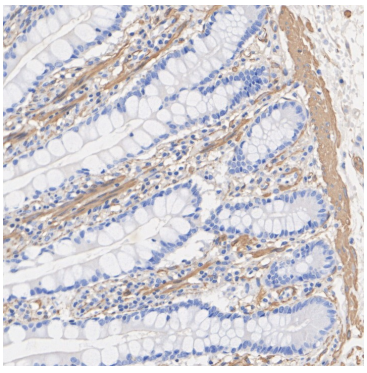

Fig2: Immunohistochemical analysis of paraffin-embedded human small intestine tissue with Mouse anti-alpha smooth muscle Actin antibody (HA610362) at 1/80,000 dilution.

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 (HA610362) at 1/80,000 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.

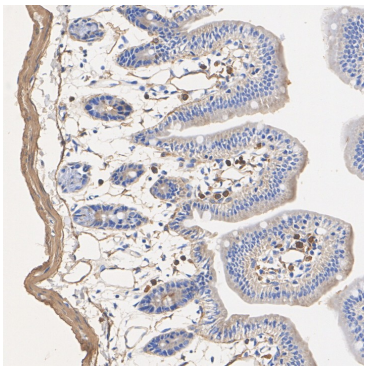

Fig3: Immunohistochemical analysis of paraffin-embedded mouse small intestine tissue with Mouse anti-alpha smooth muscle Actin antibody (HA610362) at 1/80,000 dilution.

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 (HA610362) at 1/80,000 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.

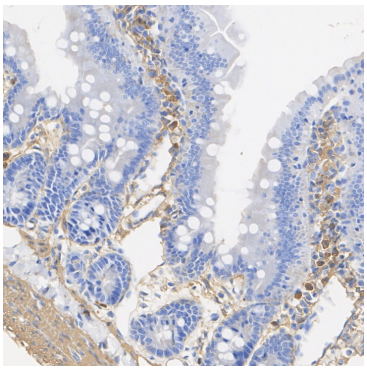

Fig4: Immunohistochemical analysis of paraffin-embedded rat small intestine tissue with Mouse anti-alpha smooth muscle Actin antibody (HA610362) at 1/80,000 dilution.

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 (HA610362) at 1/80,000 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.

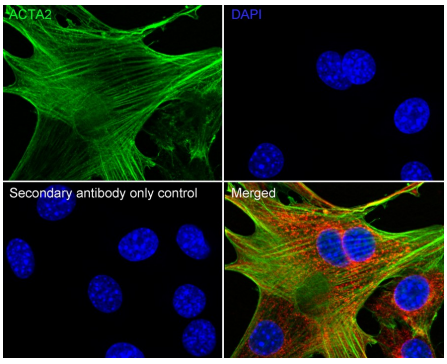

Fig5: Immunocytochemistry analysis of NIH/3T3 cells labeling alpha smooth muscle Actin with Mouse anti-alpha smooth muscle Actin antibody (HA610362) at 1/2,000 dilution.

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 Mouse anti-alpha smooth muscle Actin antibody (HA610362) at 1/2,000 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Mouse IgG H&L (iFluor™ 488, HA1125) 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 (ET1602-4, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Rabbit IgG H&L (iFluor™ 594, HA1122) were used as the secondary antibody at 1/1,000 dilution.

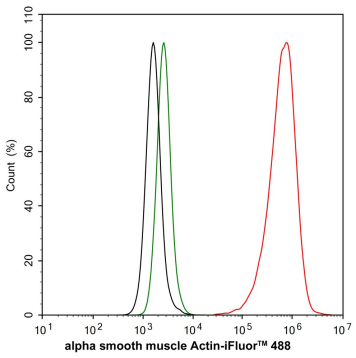

Fig6: Flow cytometric analysis of Saos-2 cells labeling alpha smooth muscle Actin.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA610362, 1/1,000) (red) compared with Mouse 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-Mouse IgG Secondary antibody (HA1125) at 1/1,000 dilution for 30 minutes at +4℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

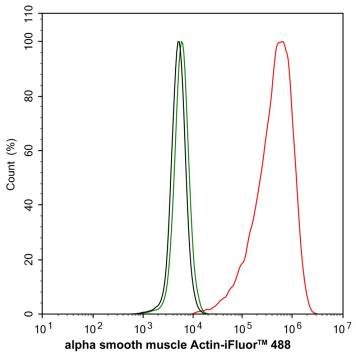

Fig7: Flow cytometric analysis of NIH/3T3 cells labeling alpha smooth muscle Actin.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA610362, 1/1,000) (red) compared with Mouse 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-Mouse IgG Secondary antibody (HA1125) at 1/1,000 dilution for 30 minutes at +4℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

Background References

1. Izumi D et al. CXCL12/CXCR4 activation by cancer-associated fibroblasts promotes integrin 1 clustering and invasiveness in gastric cancer. Int J Cancer 138:1207-19 (2016).
2. Chung SI et al. Development of a transgenic mouse model of hepatocellular carcinoma with a liver fibrosis background. BMC Gastroenterol 16:13 (2016).