abcam

Product datasheet

Anti-alpha smooth muscle Actin antibody [SP171] ab150301

KO VALIDATED Recombinant RabMAb

9 References 14 Images

Overview

Product name	Anti-alpha smooth muscle Actin antibody [SP171]
Description	Rabbit monoclonal [SP171] to alpha smooth muscle Actin
Host species	Rabbit
Tested applications	**Suitable for:** Flow Cyt (Intra), ICC/IF, mIHC, WB, IHC-P
Species reactivity	**Reacts with:** Mouse, Rat, Human **Predicted to work with:** Rabbit, Chicken, Cow, Pig
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	IHC-P: Human colon, Mouse colon, and Rat colon tissue. WB: Recombinant Human alpha smooth muscle Actin protein (**ab114148**); HeLa cell lysate. Flow Cyt (Intra): HeLa, NIH/3T3 and C6 cells. ICC/IF: NIH/3T3, SV40LT-SMC, and HeLa cells.
General notes	This product is a recombinant monoclonal antibody, which offers several advantages including: - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production For more information **see here**. **This product is FOR RESEARCH USE ONLY. For commercial use, please contact partnerships@abcam.com.**

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.60 Preservative: 0.1% Sodium azide Constituents: PBS, 1% BSA
Purity	Protein A/G purified

Purification notes	Purified from TCS by protein A/G.
Clonality	Monoclonal
Clone number	SP171
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab150301 in the following tested applications.

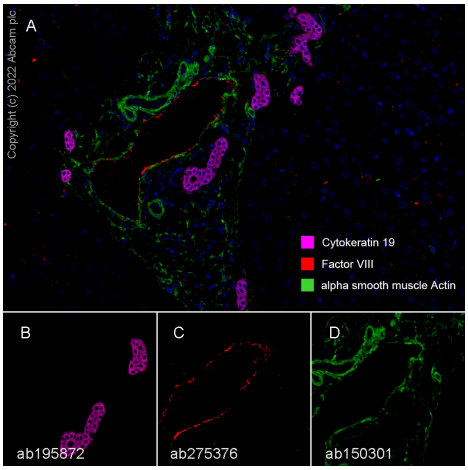
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
Flow Cyt (Intra)		Use at an assay dependent concentration.
ICC/IF		Use a concentration of 1 - 5 µg/ml.
mIHC		1/200.
WB		1/50. Predicted molecular weight: 42 kDa.
IHC-P		1/200.

Target

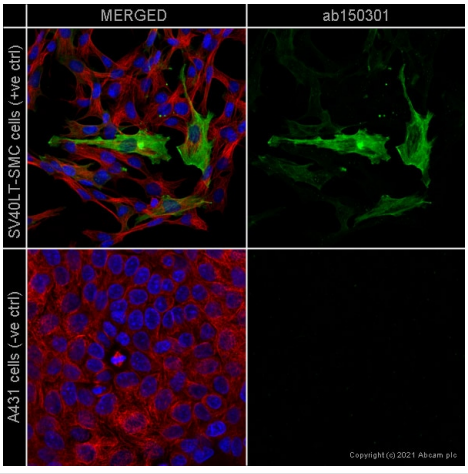
Function	Actins are highly conserved proteins that are involved in various types of cell motility and are ubiquitously expressed in all eukaryotic cells.
Involvement in disease	Defects in ACTA2 are the cause of aortic aneurysm familial thoracic type 6 (AAT6) [MIM:611788]. AATs are characterized by permanent dilation of the thoracic aorta usually due to degenerative changes in the aortic wall. They are primarily associated with a characteristic histologic appearance known as 'medial necrosis' or 'Erdheim cystic medial necrosis' in which there is degeneration and fragmentation of elastic fibers, loss of smooth muscle cells, and an accumulation of basophilic ground substance.
Sequence similarities	Belongs to the actin family.
Cellular localization	Cytoplasm > cytoskeleton.

Images



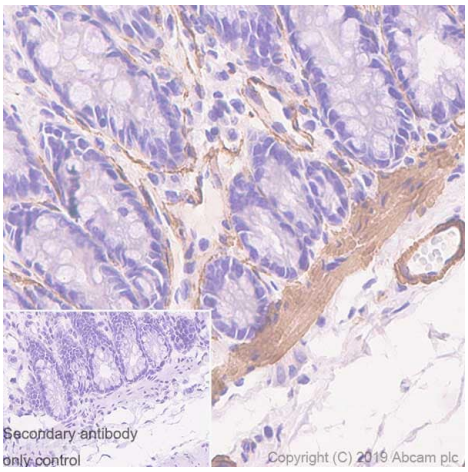
Multiplex immunohistochemistry - Anti-alpha smooth muscle Actin antibody [SP171] (ab150301)

Fluorescence multiplex immunohistochemical analysis of human liver tissue (formalin-fixed paraffin-embedded section). Panel A shows merged staining of **ab195872** anti-Cytokeratin 19 stained on branch of bile ducts (magenta; Opal™690) at 1:8000 (0.127 µg/ml) [Panel B], **ab275376** anti-Factor VIII stained on endothelial cells (red; Opal™570) at 1:1000 (0.457 µg/ml) [Panel C], and ab150301 anti-alpha smooth muscle Actin stained on smooth muscles (green; Opal™520) at 1:200 (0.14 µg/ml) [Panel C] on human liver. DAPI was used as a nuclear counter stain. Followed by Opal Polymer HRP Ms + Rb secondary. The immunostaining was performed on a Leica Biosystems BOND® RX instrument with an Opal™ 4-color kit. Image acquisition was performed with Leica SP8 confocal microscope. The section was incubated in three rounds of staining: in the order of **ab195872** for 30 mins, **ab275376** for 30 mins and ab150301 for 10 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins was used.



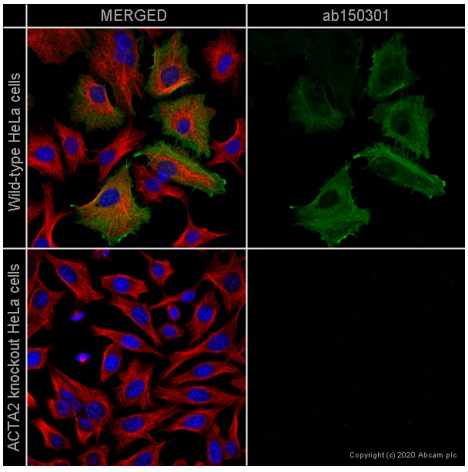
Immunocytochemistry/ Immunofluorescence - Anti-alpha smooth muscle Actin antibody [SP171] (ab150301)

ab150301 staining alpha smooth muscle Actin in SV40LT-SMC cells (positive control, top panel) and A431 cells (negative control, bottom panel). The cells were fixed with 100% methanol (5 min) then permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with ab150301 at 1µg/ml concentration and **ab7291** (Mouse monoclonal to alpha Tubulin) at 1/1000 dilution overnight at 4°C followed by a further incubation at room temperature for 1h with a goat secondary antibody to rabbit IgG (Alexa Fluor® 488) (**ab150081**) at 2 µg/ml (shown in green) and a goat secondary antibody to mouse IgG (Alexa Fluor® 594) (**ab150120**) at 2 µg/ml (shown in red). Nuclear DNA was labelled in blue with DAPI.
Image was taken with a confocal microscope (Leica-Microsystems TCS SP8).



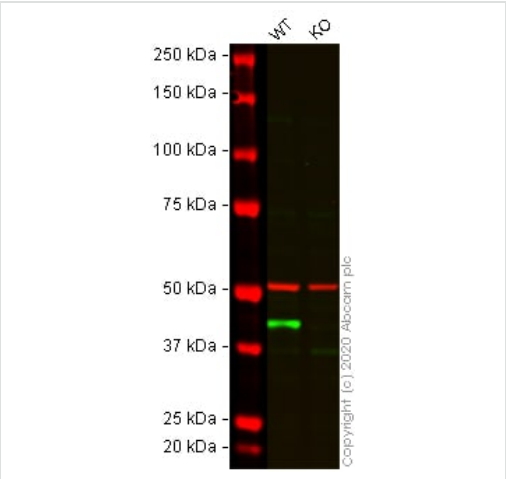
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-alpha smooth muscle Actin antibody [SP171] (ab150301)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Rat colon tissue sections labeling alpha smooth muscle Actin with ab150301 at 1/200 dilution (0.26 µg/ml). Heat mediated antigen retrieval was performed Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 10 mins. Goat Anti-Rabbit & Mouse IgG (HRP) was used as the secondary antibody. Negative control: PBS instead of the primary antibody. Hematoxylin was used as a counterstain.



Immunocytochemistry/ Immunofluorescence - Anti-alpha smooth muscle Actin antibody [SP171] (ab150301)

ab150301 staining alpha smooth muscle Actin in wild-type HeLa cells (top panel) and ACTA2 knockout HeLa cells (bottom panel). The cells were fixed with 100% methanol (5 min) then permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with ab150301 at 5µg/ml concentration and **ab7291** (Mouse monoclonal to alpha Tubulin) at 1/1000 dilution overnight at 4°C followed by a further incubation at room temperature for 1h with a goat secondary antibody to rabbit IgG (Alexa Fluor® 488) (**ab150081**) at 2 µg/ml (shown in green) and a goat secondary antibody to mouse IgG (Alexa Fluor® 594) (**ab150120**) at 2 µg/ml (shown in red). Nuclear DNA was labelled in blue with DAPI.
Image was taken with a confocal microscope (Leica-Microsystems TCS SP8).



Western blot - Anti-alpha smooth muscle Actin antibody [SP171] (ab150301)

All lanes : Anti-alpha smooth muscle Actin antibody [SP171] (ab150301) at 1/130 dilution

Lane 1 : Wild-type HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate
Lane 2 : ACTA2 knockout HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate

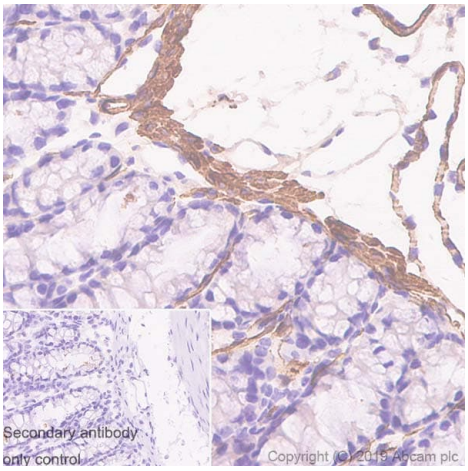
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

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

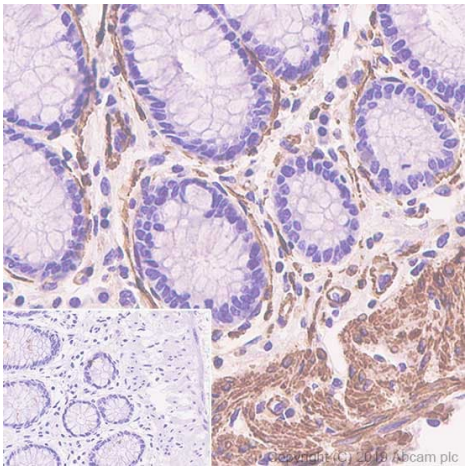
Lanes 1 - 2: Merged signal (red and green). Green - ab150301 observed at 42 kDa. Red - loading control, **ab7291** (Mouse anti-Alpha Tubulin [DM1A]) observed at 55kDa.

ab150301 was shown to react with alpha smooth muscle Actin in wild-type HeLa cells in western blot. Loss of signal was observed when ACTA2 knockout sample was used. Wild-type HeLa and ACTA2 knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3% milk in TBS-T (0.1% Tween®) before incubation with ab150301 and **ab7291** (Mouse anti-Alpha Tubulin [DM1A]) overnight at 4°C at a 1 in 130 Dilution and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



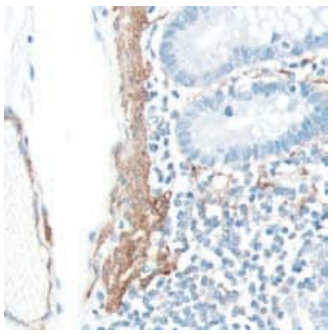
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-alpha smooth muscle Actin antibody [SP171] (ab150301)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Mouse colon tissue sections labeling alpha smooth muscle Actin with ab150301 at 1/200 dilution (0.26 µg/ml). Heat mediated antigen retrieval was performed Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 10 mins. Goat Anti-Rabbit & Mouse IgG (HRP) was used as the secondary antibody. Negative control: PBS instead of the primary antibody. Hematoxylin was used as a counterstain.



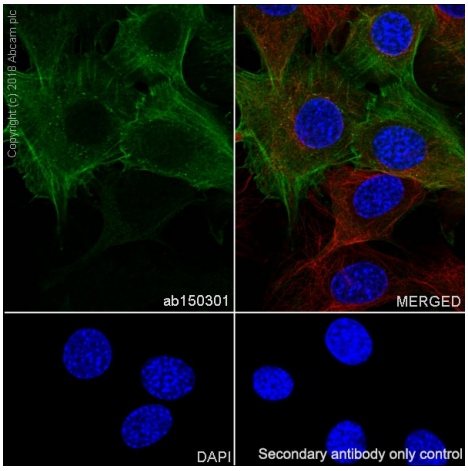
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-alpha smooth muscle Actin antibody [SP171] (ab150301)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Human colon tissue sections labeling alpha smooth muscle Actin with ab150301 at 1/200 dilution (0.26 µg/ml). Heat mediated antigen retrieval was performed Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 10 mins. Goat Anti-Rabbit & Mouse IgG (HRP) was used as the secondary antibody. Negative control: PBS instead of the primary antibody. Hematoxylin was used as a counterstain.



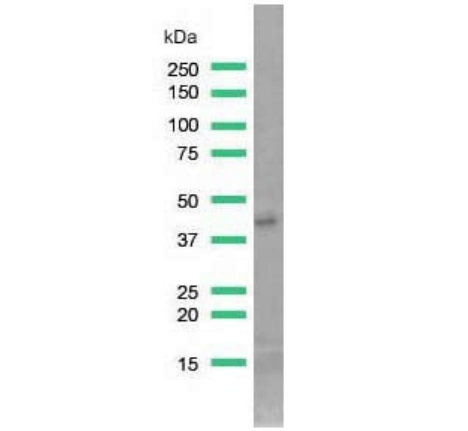
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-alpha smooth muscle Actin antibody [SP171] (ab150301)

Immunohistochemical analysis of formalin fixed, paraffin embedded Human colon tissue labelling alpha smooth muscle Actin with ab150301 at 1/200 dilution.



Immunocytochemistry/ Immunofluorescence - Anti-alpha smooth muscle Actin antibody [SP171] (ab150301)

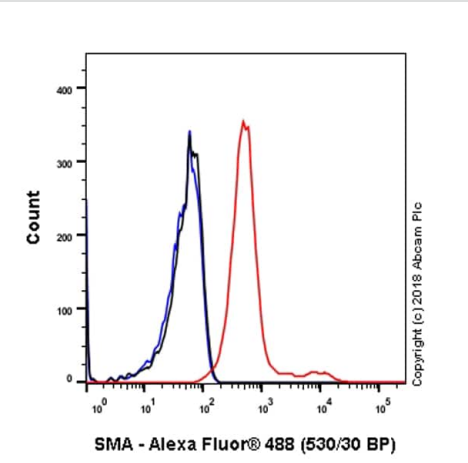
Immunocytochemistry/ Immunofluorescence analysis of NIH/3T3 (mouse embryonic fibroblast) cells labeling alpha smooth muscle Actin with purified ab150301. Cells were fixed in 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. Cells were counterstained with **ab195889** Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594). Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) was used as the secondary antibody. DAPI (blue) was used as nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.



Western blot - Anti-alpha smooth muscle Actin antibody [SP171] (ab150301)

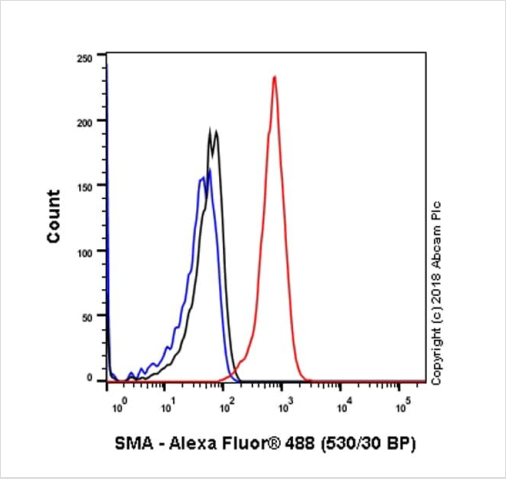
Anti-alpha smooth muscle Actin antibody [SP171] (ab150301) at 1/50 dilution + HeLa cell lysate

Predicted band size: 42 kDa



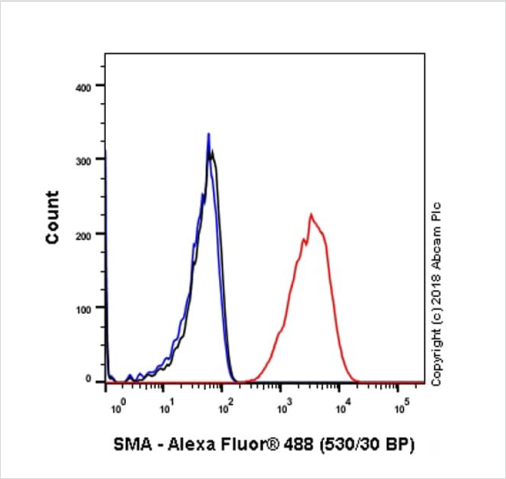
Flow Cytometry (Intracellular) - Anti-alpha smooth muscle Actin antibody [SP171] (ab150301)

Flow Cytometry analysis of HeLa (human cervix adenocarcinoma epithelial cell) cells labeling alpha smooth muscle Actin with purified ab150301 (red). Cells were fixed with 4% paraformaldehyde and permeabilised with 90% methanol. A Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) was used as the secondary antibody. Isotype control - Rabbit monoclonal IgG (**ab172730**) / Black. Unlabeled control - Unlabelled cells / blue.



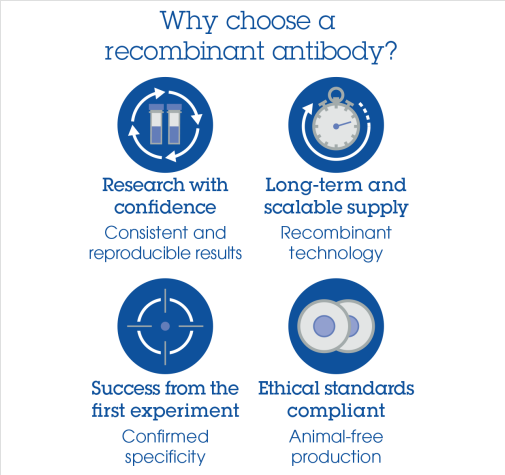
Flow Cytometry (Intracellular) - Anti-alpha smooth muscle Actin antibody [SP171] (ab150301)

Flow Cytometry analysis of C6 (Rat glial tumor glial cell) cells labeling alpha smooth muscle Actin with purified ab150301 (red). Cells were fixed with 4% paraformaldehyde and permeabilised with 90% methanol. A Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) secondary antibody was used. Isotype control - Rabbit monoclonal IgG (**ab172730**) / Black. Unlabeled control - Unlabelled cells / blue.



Flow Cytometry (Intracellular) - Anti-alpha smooth muscle Actin antibody [SP171] (ab150301)

Flow Cytometry analysis of NIH/3T3 (Mouse embryonic fibroblast) cells labeling alpha smooth muscle Actin with purified ab150301 at 1/200 dilution (0.825 µg/ml) (red). Cells were fixed with 4% paraformaldehyde and permeabilised with 90% methanol. A Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) secondary antibody was used at 1/2000 dilution. Isotype control - Rabbit monoclonal IgG (**ab172730**) / Black. Unlabeled control - Unlabelled cells / blue.



Anti-alpha smooth muscle Actin antibody [SP171] (ab150301)

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