abcam

Product datasheet

Anti-alpha smooth muscle Actin antibody [EPR5368] - BSA and Azide free ab220795

KO VALIDATED Recombinant RabMAb

★★★★★ 2 Abreviews 24 Images

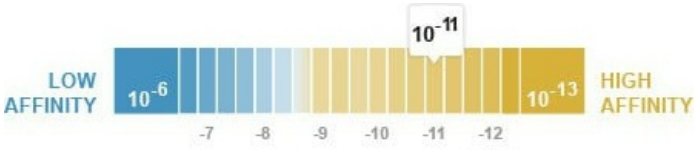
Overview

Product name	Anti-alpha smooth muscle Actin antibody [EPR5368] - BSA and Azide free
Description	Rabbit monoclonal [EPR5368] to alpha smooth muscle Actin - BSA and Azide free
Host species	Rabbit
Tested applications	**Suitable for:** ICC/IF, Flow Cyt (Intra), WB, IHC-P
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: HeLa, HEK-293, U937, SV40LT-SMC, A549, C2C12, A431 and NIH/3T3 cell lysates. Mouse and rat brain and heart tissue lysates. Human heart, skeletal muscle and lung tissue lysates. IHC-P: Human prostatic carcinoma, stomach carcinoma, tonsil, heart, skeletal muscle (exhibits vascular smooth muscle staining), normal stomach, liver, colon, tonsil and ovary tissues; Mouse Uterus and stomach tissue. ICC/IF: A-673 and HeLa cells. Flow Cyt (intra): Jurkat cells.
General notes	ab220795 is the carrier-free version of **ab124964**. Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications. Use our **conjugation kits** for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold. This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc. 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**.

Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C. Do Not Freeze.
Dissociation constant (K_D)	$K_D = 2.20 \times 10^{-11}$ M



Learn more about K_D

Storage buffer	pH: 7.2 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR5368
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab220795 in the following tested applications.

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

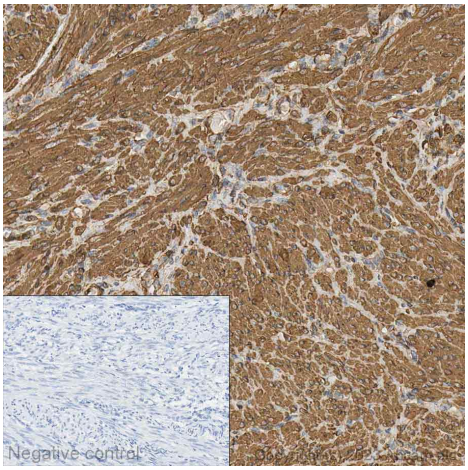
Application	Abreviews	Notes
ICC/IF		Use at an assay dependent concentration.
Flow Cyt (Intra)		Use at an assay dependent concentration. **ab199376** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
WB		Use at an assay dependent concentration. Detects a band of approximately 42 kDa (predicted molecular weight: 42 kDa).
IHC-P	★★★★★ (1)	Use at an assay dependent concentration. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol. See **IHC antigen retrieval protocols**.

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.

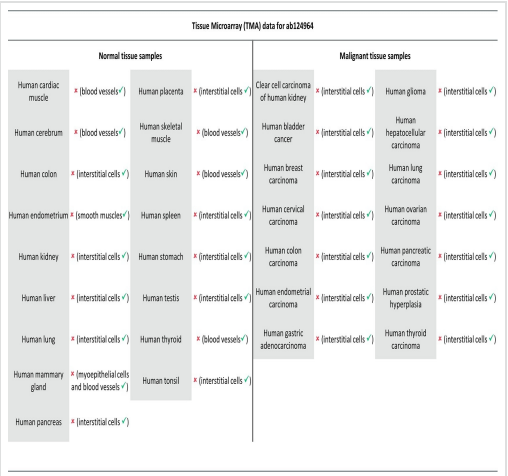
Images



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-alpha smooth muscle Actin antibody [EPR5368] - BSA and Azide free (ab220795)

Immunohistochemical analysis of formalin-fixed paraffin-embedded human endometrium labelling alpha smooth muscle actin with **ab124964** at a concentration of 0.07µg/ml. The immunostaining was performed on a Ventana DISCOVERY ULTRA (Roche Tissue Diagnostics) instrument with an OptiView DAB IHC Detection Kit. Heat mediated antigen retrieval was conducted for 32min with ULTRA cell conditioning solution (CC1 pH8.5). **ab124964** anti alpha smooth muscle actin antibody was incubated at 37°C for 16min. Sections were counterstained with Hematoxylin II. Image inset shows absence of staining in secondary antibody only control.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab124964**).

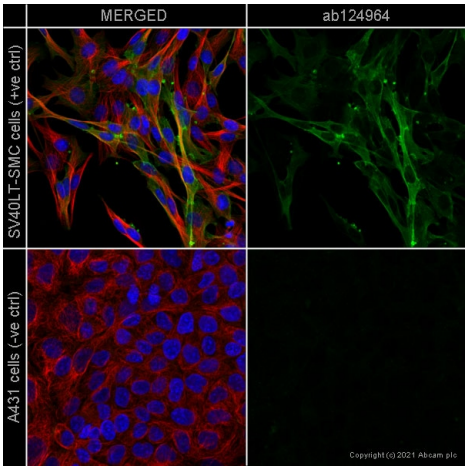


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-alpha smooth muscle Actin antibody [EPR5368] - BSA and Azide free (ab220795)

This data was developed using the same antibody clone in a different buffer formulation (**ab124964**).

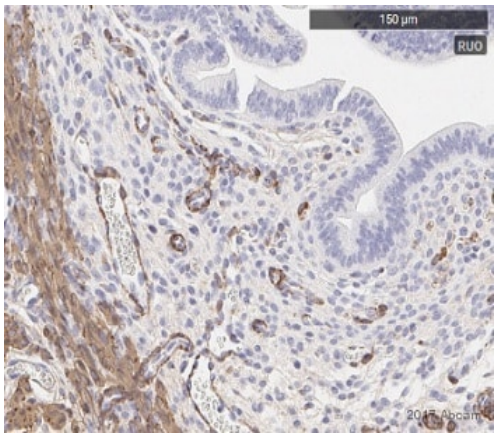
Tissue Microarrays stained for Anti-alpha smooth muscle Actin antibody [EPR5368] using **ab124964** in immunohistochemical analysis. This table provides a detailed overview of positive (tick mark) and negaive (cross mark) staining per sample type tested.
The section was incubated with **ab124964** for 30 mins at room temperature followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**).
The immunostaining was performed on a Leica Biosystems BOND® RX instrument.

Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins.



Immunocytochemistry/ Immunofluorescence - Anti-alpha smooth muscle Actin antibody [EPR5368] - BSA and Azide free (ab220795)

This data was developed using the same antibody clone in a different buffer formulation (**ab124964**). **ab124964** 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 **ab124964** at 0.5µg/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).

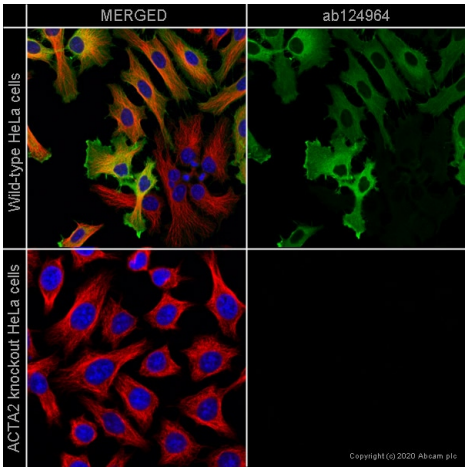


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-alpha smooth muscle Actin antibody [EPR5368] - BSA and Azide free (ab220795)

This image is courtesy of an anonymous Abreview.

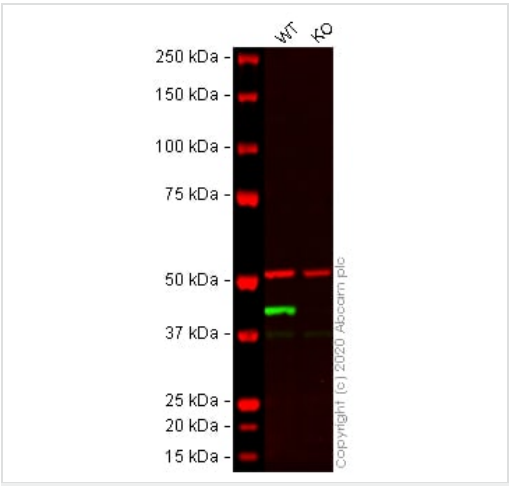
ab124964 staining alpha smooth muscle Actin in Mouse Uterus tissue sections by Immunohistochemistry (Formalin/PFA perfusion fixed frozen sections). Tissue samples were fixed by perfusion with formaldehyde, blocked with PB **ab64226** for 10 minutes at Room temperature and antigen retrieval was by heat mediation in citrate buffer. The sample was incubated with primary antibody (1/2000) for 30 minutes. A HRP-conjugated Goat anti-rabbit polyclonal (undiluted) was used as the secondary antibody.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab124964**).



Immunocytochemistry/ Immunofluorescence - Anti-alpha smooth muscle Actin antibody [EPR5368] - BSA and Azide free (ab220795)

This data was developed using the same antibody clone in a different buffer formulation (**ab124964**). **ab124964** 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 **ab124964** at 1/500 dilution 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 [EPR5368] - BSA and Azide free (ab220795)

All lanes : Anti-alpha smooth muscle Actin antibody [EPR5368] (**ab124964**) at 1/10000 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.

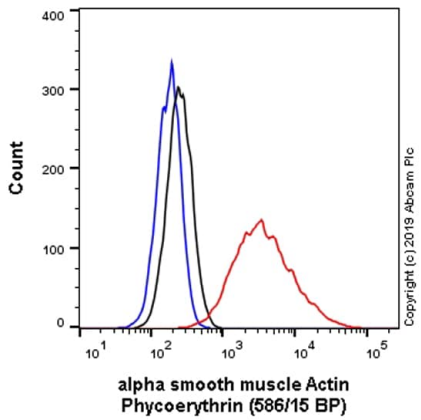
Performed under reducing conditions.

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

This data was developed using the same antibody clone in a different buffer formulation (**ab124964**).

Lanes 1 - 2: Merged signal (red and green). Green - **ab124964** observed at 42 kDa. Red - loading control, **ab7291** (Mouse anti-Alpha Tubulin [DM1A]) observed at 55kDa.

ab124964 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 **ab124964** and **ab7291** (Mouse anti-Alpha Tubulin [DM1A]) overnight at 4°C at a 1 in 10000 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.



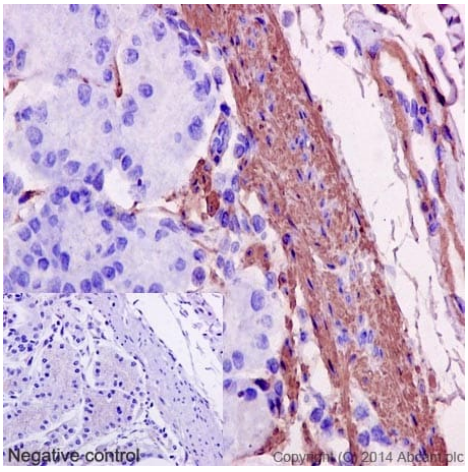
Flow Cytometry (Intracellular) - Anti-alpha smooth muscle Actin antibody [EPR5368] - BSA and Azide free (ab220795)

Clone EPR5368 (ab220795) has been successfully conjugated by Abcam. This image was generated using Anti-alpha smooth muscle Actin antibody [EPR5368] (PE). Please refer to **ab208844** for protocol details.

Overlay histogram showing SV40LT-SMC cells stained with **ab208844** (red line). The cells were fixed with 4% formaldehyde (10 min) and then permeabilized with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS / 10% normal goat serum to block non-specific protein-protein interactions followed by the antibody (**ab208844**, 1/5000 dilution) for 30 min at 22°C.

Isotype control antibody (black line) was Rabbit IgG (monoclonal) Phycoerythrin (**ab209478**) used at the same concentration and conditions as the primary antibody. Unlabelled sample (blue line) was also used as a control.

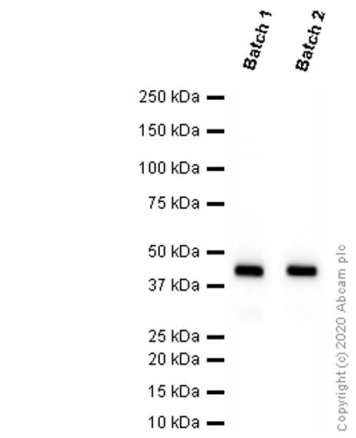
Acquisition of >5,000 events were collected using a 50 mW Yellow/Green laser (561nm) and 586/15 bandpass filter.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-alpha smooth muscle Actin antibody [EPR5368] - BSA and Azide free (ab220795)

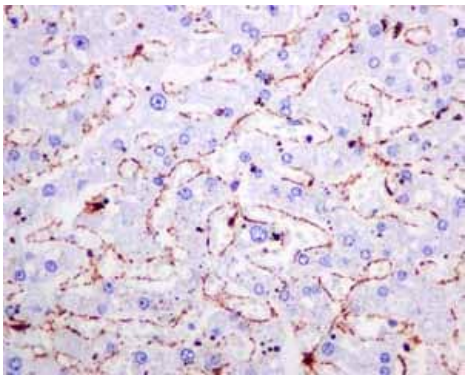
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human stomach tissue labelling alpha smooth muscl Actin with purified **ab124964** at 1/1000. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. A prediluted HRP-polymer conjugated anti-rabbit IgG was used as the secondary antibody. Negative control using PBS instead of primary antibody. Counterstained with Hematoxylin.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab124964**).



Western blot - Anti-alpha smooth muscle Actin antibody [EPR5368] - BSA and Azide free (ab220795)

This data was developed using **ab124964**, the same antibody clone in a different buffer formulation. Different batches of **ab124964** were tested on HEK-293 (Human embryonic kidney epithelial cell) lysate at 2.1 µg/ml. 15 µg of lysate was loaded in each lane. Bands observed at 42 kDa.

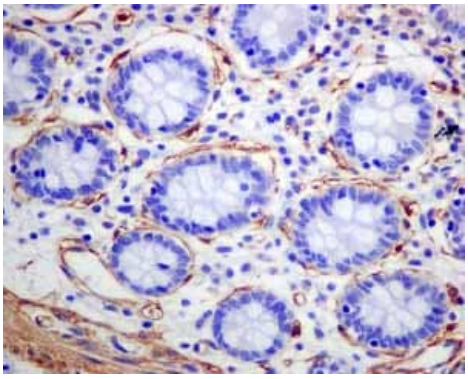


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-alpha smooth muscle Actin antibody [EPR5368] - BSA and Azide free (ab220795)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of normal human liver vessels tissue labelling alpha smooth muscle Actin with unpurified **ab124964**.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab124964**).

Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

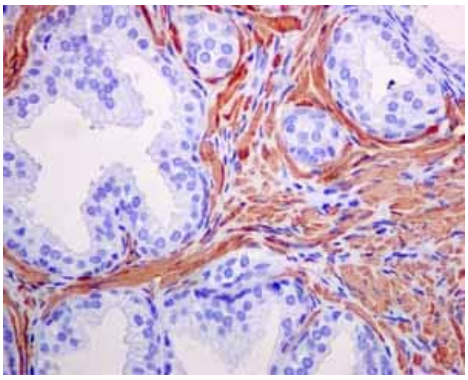


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-alpha smooth muscle Actin antibody [EPR5368] - BSA and Azide free (ab220795)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of normal human colon smooth muscle tissue labelling alpha smooth muscle Actin with unpurified **ab124964**.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab124964**).

Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

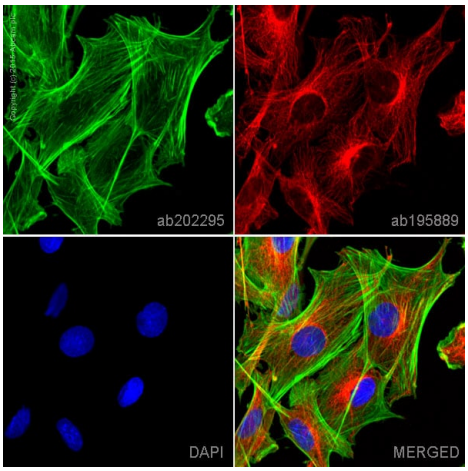


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-alpha smooth muscle Actin antibody [EPR5368] - BSA and Azide free (ab220795)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human prostatic carcinoma smooth muscles tissue labelling alpha smooth muscle Actin with unpurified **ab124964**.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab124964**).

Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.



Immunocytochemistry/ Immunofluorescence - Anti-alpha smooth muscle Actin antibody [EPR5368] - BSA and Azide free (ab220795)

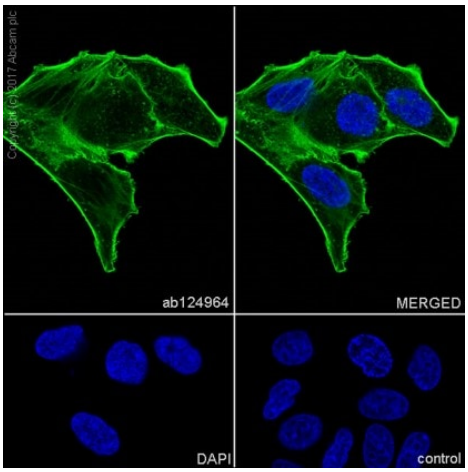
Clone EPR5368 (ab220795) has been successfully conjugated by Abcam. This image was generated using Anti-alpha smooth muscle Actin antibody [EPR5368] (Alexa Fluor® 488). Please refer to **ab202295** for protocol details.

ab202295 staining alpha smooth muscle Actin in SV40LT-SMC cells. The cells were fixed with 4% formaldehyde (10 min), 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 overnight at +4°C with ab at a 1/100 dilution (shown in green) and **ab195889**, Mouse monoclonal to alpha Tubulin (Alexa Fluor® 594), at a 1/250 dilution (shown in red). Nuclear DNA was labelled with DAPI (shown in blue).

Image was taken with a confocal microscope (Leica-Microsystems,

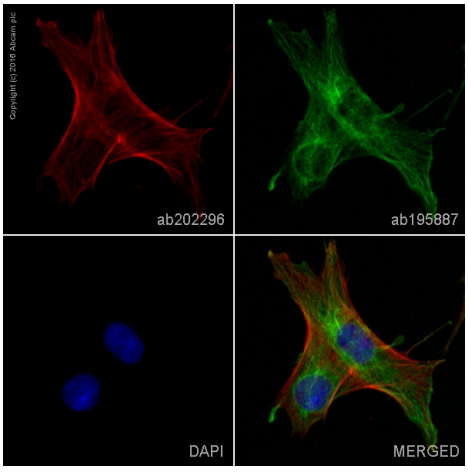
TCS SP8).

This product also gave a positive signal under the same testing conditions in SV40LT-SMC cells fixed with 100% methanol (5 min)



Immunocytochemistry/ Immunofluorescence - Anti-alpha smooth muscle Actin antibody [EPR5368] - BSA and Azide free (ab220795)

Immunocytochemistry/Immunofluorescence analysis of HeLa cells labeling alpha smooth muscle Actin with ab220795 at 1/500. Cells were fixed with 4% paraformaldehyde and permeabilised with 0.1% tritonX-100. An Goat anti rabbit IgG (Alexa Fluor® 488) **ab150077** (1/1000) was used as the secondary antibody. DAPI (blue) was used as the nuclear counterstain.



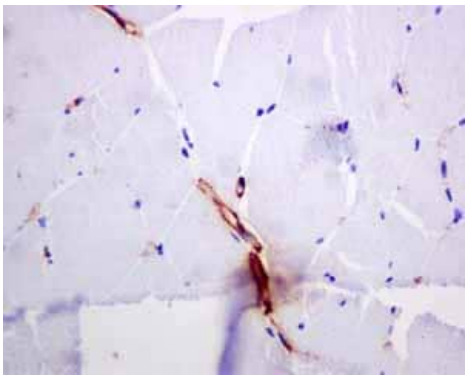
Immunocytochemistry/ Immunofluorescence - Anti-alpha smooth muscle Actin antibody [EPR5368] - BSA and Azide free (ab220795)

Clone EPR5368 (ab220795) has been successfully conjugated by Abcam. This image was generated using Anti-alpha smooth muscle Actin antibody [EPR5368] (Alexa Fluor® 647). Please refer to **ab202296** for protocol details.

ab202296 staining alpha smooth muscle Actin in SV40LT-SMC cells. The cells were fixed with 4% formaldehyde (10 min), 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 overnight at +4°C with **ab202296** at 1/5000 dilution (shown in red) and **ab195887**, Mouse monoclonal to alpha Tubulin (Alexa Fluor® 488), at 1/250 dilution (shown in green). Nuclear DNA was labelled with DAPI (shown in blue).

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

This product also gave a positive signal under the same testing conditions in SV40LT-SMC cells fixed with 100% methanol (5min).

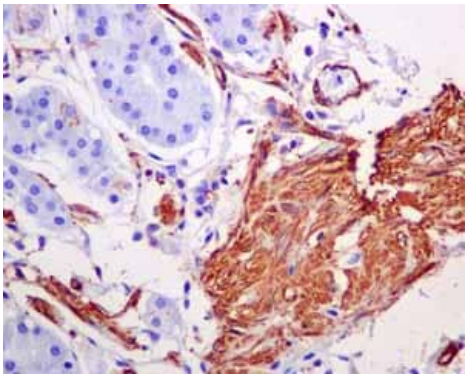


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-alpha smooth muscle Actin antibody [EPR5368] - BSA and Azide free (ab220795)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis showing vascular smooth muscle staining in skeletal muscle tissue using alpha smooth muscle Actin with unpurified **ab124964**. Note positive staining on smooth muscle cells but negative on striated muscle cells.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab124964**).

Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

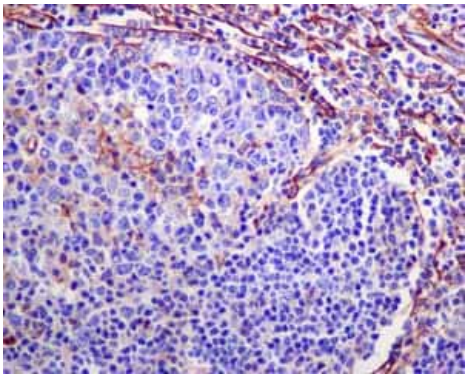


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-alpha smooth muscle Actin antibody [EPR5368] - BSA and Azide free (ab220795)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human stomach carcinoma smooth muscles tissue labelling alpha smooth muscle Actin with unpurified **ab124964**.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab124964**).

Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

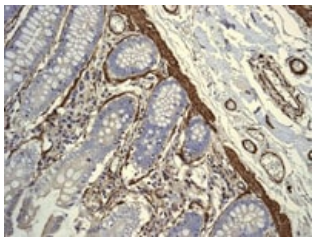


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-alpha smooth muscle Actin antibody [EPR5368] - BSA and Azide free (ab220795)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of normal human tonsil vessels tissue labelling alpha smooth muscle Actin with unpurified **ab124964**.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab124964**).

Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

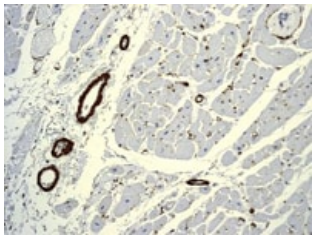


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-alpha smooth muscle Actin antibody [EPR5368] - BSA and Azide free (ab220795)

This IHC data was generated using the same anti-alpha smooth muscle Actin antibody clone, EPR5368, in a different buffer formulation (cat# **ab124964**).

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human ovary tissue labelling alpha smooth muscle Actin with unpurified **ab124964** at 1/1000 dilution.

Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

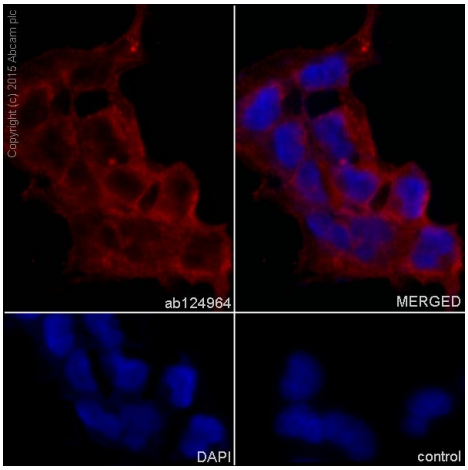


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-alpha smooth muscle Actin antibody [EPR5368] - BSA and Azide free (ab220795)

This IHC data was generated using the same anti-alpha smooth muscle Actin antibody clone, EPR5368, in a different buffer formulation (cat# **ab124964**).

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human heart tissue labelling alpha smooth muscle actin with unpurified **ab124964** at 1/1000 dilution. Note positive staining on smooth muscle cells but negative on striated muscle cells.

Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

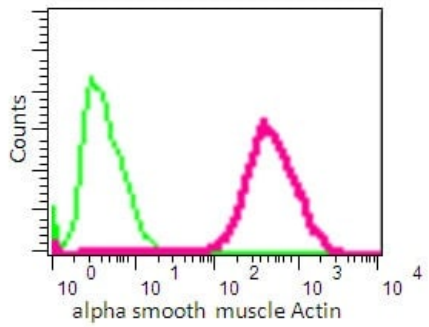


Immunocytochemistry/ Immunofluorescence - Anti-alpha smooth muscle Actin antibody [EPR5368] - BSA and Azide free (ab220795)

Immunocytochemistry/Immunofluorescence analysis of A-673 (human muscle Ewing's Sarcoma cell line) cells labelling alpha smooth muscle Actin with purified **ab124964** at 1/300. Cells were fixed with 4% paraformaldehyde. An Alexa Fluor® 555-conjugated goat anti-rabbit IgG (1/500) was used as the secondary antibody. DAPI (blue) was used as the nuclear counterstain.

Control: primary antibody (1/300) and secondary antibody, **ab150113**, an Alexa Fluor® 488-conjugated goat anti-mouse IgG (1/500).

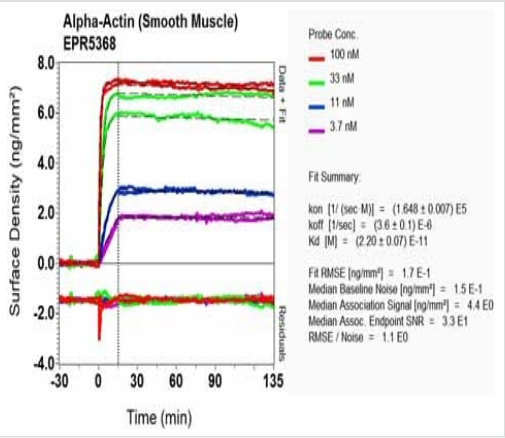
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab124964**).



Flow Cytometry (Intracellular) - Anti-alpha smooth muscle Actin antibody [EPR5368] - BSA and Azide free (ab220795)

Intracellular Flow Cytometry analysis of Jurkat (human T cell leukemia cell line from peripheral blood) cells labelling alpha smooth muscle Actin with purified **ab124964** at 1/30 (red). Cells were fixed with 2% paraformaldehyde. A FITC-conjugated goat anti-rabbit IgG (1/150) was used as the secondary antibody. Green - Isotype control, rabbit monoclonal IgG.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab124964**).



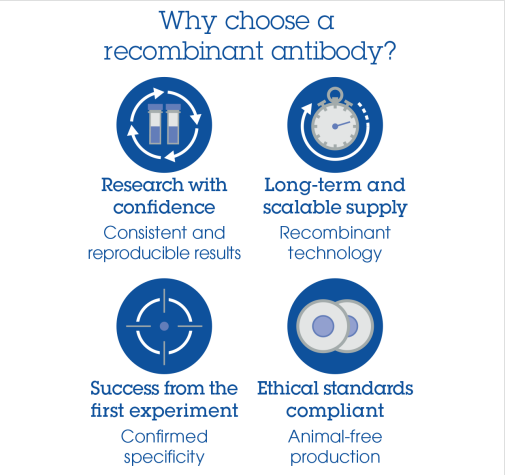
OI-RD Scanning - Anti-alpha smooth muscle Actin antibody [EPR5368] - BSA and Azide free (ab220795)

Equilibrium disassociation constant (K_D)

Learn more about K_D

Click here to learn more about K_D

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab124964**).



Anti-alpha smooth muscle Actin antibody [EPR5368] - BSA and Azide free (ab220795)

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