

Anti-alpha smooth muscle Actin antibody [EPR5368]

Anti-alpha smooth muscle Actin antibody [EPR5368] (ab124964) is a rabbit monoclonal antibody detecting alpha smooth muscle Actin in **Western Blot, Flow Cytometry (Intra), IHC-P, ICC/IF**. Suitable for **Human, Mouse, Rat**.

- KO validated for confirmed specificity
- Biophysical QC for unrivalled batch-batch consistency
- Over 500 publications

Chicken and goat version of this clone also available as ab321866 and ab320058. Same clone, same staining pattern, different species antibody backbone.
Learn more about chimeric antibodies

Recombinant

RabMAb

KO Validated

20ul selling size

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Clone number	EPR5368
Purification technique	Affinity purification Protein A
Dissociation constant	2.2 x 10 ⁻¹¹ M
Concentration	0.151 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IHC-P

Tested

Species	Mouse
Dilution info	1/1000 - 1/2500
Notes	Perform heat-mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

Species	Human
Dilution info	1/1000 - 1/2500
Notes	Perform heat-mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

Expected

Species	Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

WB

Tested

Species	Mouse
Dilution info	1/10000 - 1/50000
Notes	For unpurified use at 1/1000.

Species	Rat
Dilution info	1/10000 - 1/50000
Notes	For unpurified use at 1/1000.

Species	Human
Dilution info	1/10000 - 1/50000

Notes

For unpurified use at 1/1000.

ICC/IF

Tested

Species	Human
Dilution info	1/250 - 1/500
Notes	-

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	1/100 - 1/200
Notes	ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Target data

[See full target information ACTA2](#) [↗](#)

Function	Actins are highly conserved proteins that are involved in various types of cell motility and are ubiquitously expressed in all eukaryotic cells.
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Storage

Shipped at conditions	Conditional Ambient
Appropriate short-term storage duration	1-2 weeks
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C
Aliquoting information	Upon delivery aliquot
Storage information	Avoid freeze / thaw cycle

Notes

Anti-alpha smooth muscle Actin antibody [EPR5368] (ab124964) was developed by Abcam using patented rabbit monoclonal antibody technology and is validated for use in Flow Cyt (Intra), ICC/IF, IHC-P and WB.

Anti-alpha smooth muscle Actin antibody [EPR5368] (ab124964) was first used in a scientific publication in 2014 and has been cited over 393 times in peer reviewed journals. It's performance in Western Blot and IHC in human, mouse and rat samples is trusted by the scientific community.

Abcam's high quality manufacturing and validation processes ensure Anti-alpha smooth muscle Actin antibody [EPR5368] (ab124964) has high sensitivity and specificity alongside high lot-to-lot consistency and reproducibility.

The specificity of Anti-alpha smooth muscle Actin antibody [EPR5368] (ab124964) has been confirmed by Western Blot testing in alpha smooth muscle Actin knockout HeLa cells.

Anti-alpha smooth muscle Actin antibody [EPR5368] (ab124964) has 20 independent reviews from customers.

alpha smooth muscle Actin antibodies are often used as loading controls in Western Blot. Anti-alpha smooth muscle Actin antibody [EPR5368] has been verified in Western Blot samples and detects a band at 42kDa Molecular weight.

Anti-alpha smooth muscle Actin antibody [EPR5368] (ab124964) specifically detects alpha smooth muscle Actin (UniProt ID: P62736; Molecular weight: 42kDa) and is sold in 100 µL and 1 mL selling sizes.

Conjugation-ready, carrier free format available for antibody clone EPR5368 - ab220795.

Antibody clone EPR5368 is also available pre-conjugated to a variety of labels for your convenience - Alexa Fluor® 488, Alexa Fluor® 647, Alexa Fluor® 555, Alexa Fluor® 594, PE, APC (ab202295, ab202296, ab202509, ab202510, ab208844, ab225143).

Alpha-Smooth Muscle Actin (α-SMA) is a critical protein involved in smooth muscle contraction, encoded by the ACTA2 gene. Predominantly found in vascular smooth muscle cells, α-SMA plays a vital role in fibrogenesis and wound healing. It is often used as a marker for myofibroblast formation, which are specialized cells that contribute to tissue

repair and fibrosis. The expression of α -SMA is associated with the activation of myofibroblasts, making it an important indicator in studies of fibrosis and other related conditions.

Patented technology

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

What are the advantages of a recombinant monoclonal antibody?

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 batch production

For more information, read more on recombinant antibodies.

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary

Alpha smooth muscle actin (α -SMA) also known as ACTA2 is an actin isoform with a specific role in the contractile function of smooth muscle cells. The molecular weight of α -SMA is approximately 42 kDa. This protein is expressed widely in vascular smooth muscle cells the peritoneal lining and myofibroblasts. It often serves as a marker for these cell types. The expression of α -SMA is critical for the mechanical attributes of cells contributing to the rigidity and contractility of tissues where it is present.

Biological function summary

Alpha smooth muscle actin aids in maintaining the structural integrity of tissues by forming part of the actin cytoskeleton an important element in cellular support. It exists in high concentration in stress fibers contributing to cellular movements and shape maintenance. These actions are essential in various dynamic cellular processes such as cell migration and adhesion. Alpha smooth muscle actin does not typically form complexes but it associates with other components of the actin cytoskeleton to ensure cell stability and function.

Pathways

Alpha smooth muscle actin functions significantly within the TGF-beta signaling pathway which influences cell proliferation differentiation and apoptosis. It interacts closely with proteins such as myosin to facilitate cellular contractility and motility. Additionally α -SMA plays a part in the RhoA/Rho kinase (ROCK) pathway connecting with regulators of actin filament organization. These pathways are essential for modulation of smooth muscle contraction and actin filament assembly contributing to vascular development and wound healing.

Associated diseases and disorders

Alpha smooth muscle actin is importantly involved in fibrotic diseases and vascular diseases like arteriosclerosis. Increased expression of α -SMA is often observed in fibrotic tissues contributing to pathogenesis due to excessive extracellular matrix deposition. In the context of vascular disorders overexpression of α -SMA can lead to abnormal vascular remodeling often seen in diseases like hypertension. Interactions with proteins such as fibronectin and collagens facilitate these pathological changes highlighting the importance of α -SMA in disease development.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

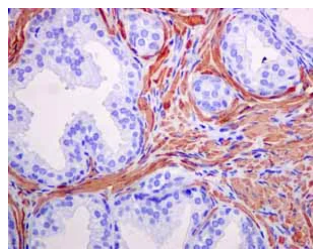
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
[Terms & Conditions.](#)

25 product images

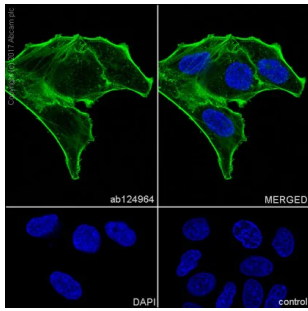


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-alpha smooth muscle Actin antibody [EPR5368] (ab124964)

Alpha smooth muscle Actin Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-alpha smooth muscle Actin antibody

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

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

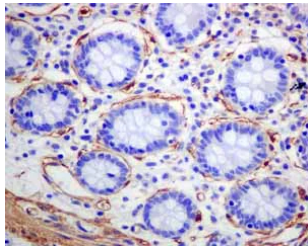


Immunocytochemistry/ Immunofluorescence - Anti-alpha smooth muscle Actin antibody [EPR5368] (ab124964)

Alpha smooth muscle Actin Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-alpha smooth muscle Actin antibody

Immunocytochemistry/Immunofluorescence analysis of HeLa cells labeling alpha smooth muscle Actin with ab124964 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.

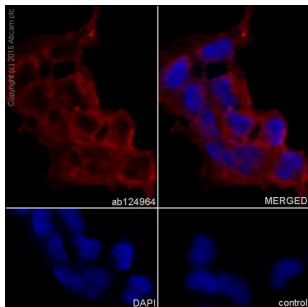


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-alpha smooth muscle Actin antibody [EPR5368] (ab124964)

Alpha smooth muscle Actin Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-alpha smooth muscle Actin antibody

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

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

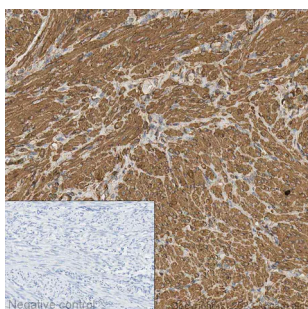


Immunocytochemistry/ Immunofluorescence - Anti-alpha smooth muscle Actin antibody [EPR5368] (ab124964)

Alpha smooth muscle Actin Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-alpha smooth muscle Actin antibody

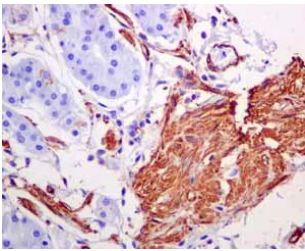
Immunocytochemistry/Immunofluorescence analysis of A-673 (human muscle Ewing's Sarcoma cell line) cells labelling alpha smooth muscle Actin with purified ab124964 at 1/300. Cells were fixed with 4% paraformaldehyde. An Alexa Fluor® 555-conjugated goat anti-rabbit IgG (1/500) was used as the secondary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



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-alpha smooth muscle Actin antibody [EPR5368] (ab124964)

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.

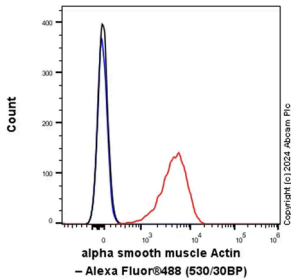


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-alpha smooth muscle Actin antibody [EPR5368] (ab124964)

Alpha smooth muscle Actin Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-alpha smooth muscle Actin antibody

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

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



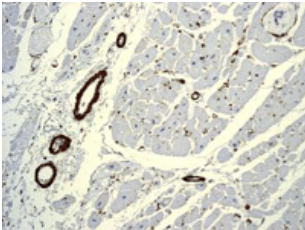
Flow Cytometry (Intracellular) - Anti-alpha smooth muscle Actin antibody [EPR5368] (ab124964)

Alpha smooth muscle Actin Flow Cytometry (Intracellular) staining of Jurkat (human T cell leukemia T lymphocyte from peripheral blood) using rabbit Anti-alpha smooth muscle Actin antibody

Flow cytometry overlay histogram showing Jurkat cells (human T cell leukemia T lymphocyte from peripheral blood) fixed with 4% paraformaldehyde and permeabilised with 90% methanol, stained with ab124964 at 1/150 dilution (0.1 µg) red line. Goat Anti-Rabbit IgG (Alexa Fluor® 488, [ab150081](#)) secondary antibody used at a 1/5000 dilution.

Isotype control: Rabbit monoclonal IgG ([ab172730](#)) black line.

Unlabelled control: Cells without incubation with primary antibody and secondary antibody blue line.

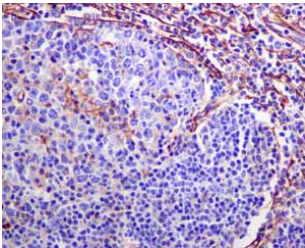


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-alpha smooth muscle Actin antibody [EPR5368] (ab124964)

Alpha smooth muscle Actin Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-alpha smooth muscle Actin antibody

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.

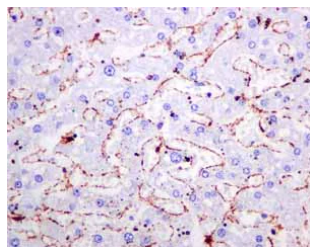
Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-alpha smooth muscle Actin antibody [EPR5368] (ab124964)

Alpha smooth muscle Actin Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-alpha smooth muscle Actin antibody

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of normal human tonsil vessels tissue labelling alpha smooth muscle Actin with unpurified ab124964. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

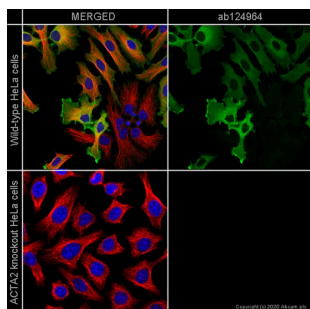


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-alpha smooth muscle Actin antibody [EPR5368] (ab124964)

Alpha smooth muscle Actin Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-alpha smooth muscle Actin antibody

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

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

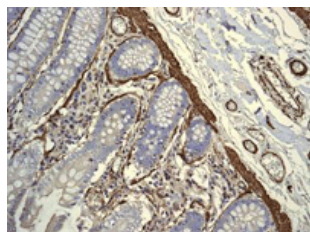


Immunocytochemistry/ Immunofluorescence - Anti-alpha smooth muscle Actin antibody [EPR5368] (ab124964)

Alpha smooth muscle Actin Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-alpha smooth muscle Actin antibody

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.

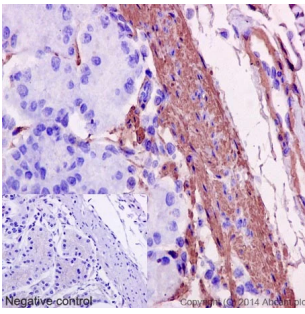
Image was taken with a confocal microscope (Leica-Microsystems TCS SP8).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-alpha smooth muscle Actin antibody [EPR5368] (ab124964)

Alpha smooth muscle Actin Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-alpha smooth muscle Actin antibody

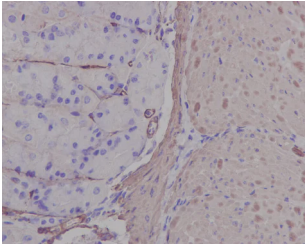
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human ovary tissue labelling alpha smooth muscle Actin with unpurified ab124964 at 1/1000 dilution. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-alpha smooth muscle Actin antibody [EPR5368] (ab124964)

Alpha smooth muscle Actin Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-alpha smooth muscle Actin antibody

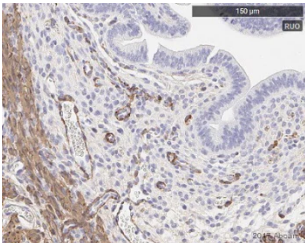
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human stomach tissue labelling alpha smooth muscle Actin with purified ab124964 at 1/1000. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. A prediluted HRP-polymer conjugated anti-rabbit IgG was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



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-alpha smooth muscle Actin antibody [EPR5368] (ab124964)

Alpha smooth muscle Actin Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-alpha smooth muscle Actin antibody

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of mouse stomach tissue labelling alpha smooth muscle Actin with 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. Counterstained with Hematoxylin. Positive staining on smooth muscle in mouse stomach.

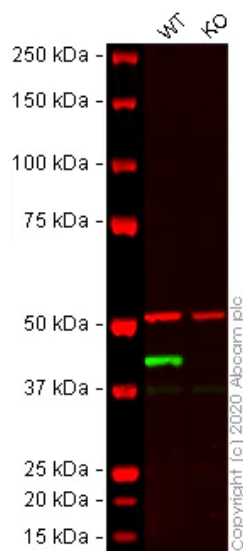


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-alpha smooth muscle Actin antibody [EPR5368] (ab124964)

Alpha smooth muscle Actin Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-alpha smooth muscle Actin antibody

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 image is courtesy of an anonymous customer review.



Western blot - Anti-alpha smooth muscle Actin antibody [EPR5368] (ab124964)

Alpha smooth muscle Actin Western blot staining using rabbit Anti-alpha smooth muscle Actin antibody

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.

All lanes:

Western blot - 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 at 20 µg

Lane 2:

Western blot - Human ACTA2 knockout HeLa cell lysate ([ab264499](#)) at 20 µg

Performed under reducing conditions.

Predicted band size: 42 kDa

Observed band size: 42 kDa



Western blot - Anti-alpha smooth muscle Actin antibody [EPR5368] (ab124964)

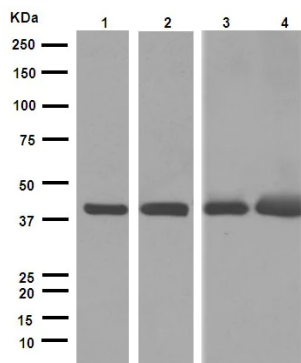
Alpha smooth muscle Actin Western blot staining using rabbit Anti-alpha smooth muscle Actin antibody

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.

All lanes:

Western blot - Anti-alpha smooth muscle Actin antibody [EPR5368] (ab124964)

Predicted band size: 42 kDa



Western blot - Anti-alpha smooth muscle Actin antibody [EPR5368] (ab124964)

Blocking buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM /TBST.

All lanes:

Western blot - Anti-alpha smooth muscle Actin antibody [EPR5368] (ab124964) at 1/10000 dilution

Lane 1:

HeLa (human epithelial cell line from cervix adenocarcinoma) cell lysate at 20 µg

Lane 2:

HEK-293 (human epithelial cell line from embryonic kidney) cell lysate at 20 µg

Lane 3:

U937 (human histiocytic lymphoma cell line) cell lysate at 20 µg

Lane 4:

A431 (human epidermoid carcinoma cell line) cell lysate at 20 µg

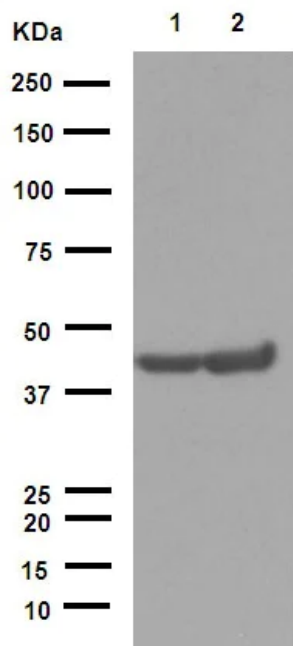
Secondary

All lanes:

Peroxidase-conjugated goat anti-rabbit IgG (H+L) at 1/1000 dilution

Predicted band size: 42 kDa, 45 kDa, 75 kDa

Observed band size: 42 kDa, 45 kDa, 60-72 kDa



Western blot - Anti-alpha smooth muscle Actin antibody [EPR5368] (ab124964)

Alpha smooth muscle Actin Western blot staining using rabbit Anti-alpha smooth muscle Actin antibody

Blocking buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM /TBST.

All lanes:

Western blot - Anti-alpha smooth muscle Actin antibody [EPR5368] (ab124964) at 1/50000 dilution

Lane 1:

Mouse brain tissue lysate at 20 µg

Lane 2:

Rat brain tissue lysate at 20 µg

Secondary

All lanes:

Peroxidase-conjugated goat anti-rabbit IgG (H+L) at 1/1000 dilution

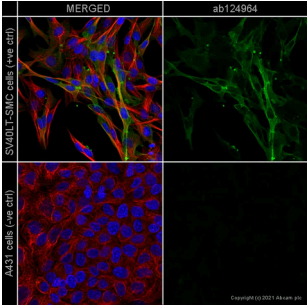
Predicted band size: 42 kDa

Observed band size: 42 kDa

Tissue Microarrays (TMA) data for ab124964									
Normal tissue samples					Abnormal tissue samples				
Sample ID	Sample Type	Sample Source	Sample Size (mm ²)	Sample Weight (mg)	Sample ID	Sample Type	Sample Source	Sample Size (mm ²)	Sample Weight (mg)
SV40LT-SMC cells (+ve ctrl)	Cell Culture	SV40LT-SMC cells	10	10	SV40LT-SMC cells (+ve ctrl)	Cell Culture	SV40LT-SMC cells	10	10
A431 cells (-ve ctrl)	Cell Culture	A431 cells	10	10	A431 cells (-ve ctrl)	Cell Culture	A431 cells	10	10

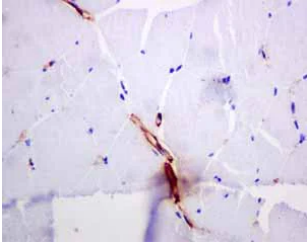
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-alpha smooth muscle Actin antibody [EPR5368] (ab124964)

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 negative (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] (ab124964)

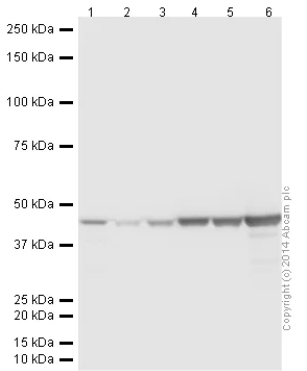
ab124964 staining alpha smooth muscle Actin in SV40LT-SMC cells (positive control, top panel) and A431 cells (negative control, bottom panel). The cells were fixed with 100% methanol (5 min) then permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M 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] (ab124964)

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.

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



Western blot - Anti-alpha smooth muscle Actin antibody [EPR5368] (ab124964)

Alpha smooth muscle Actin Western blot staining using rabbit Anti-alpha smooth muscle Actin antibody

This blot was produced using a 4-12% Bis-tris gel under the MOPS buffer system. The gel was run at 200V for 50 minutes before being transferred onto a Nitrocellulose membrane at 30V for 70 minutes. The membrane was then blocked for an hour using 2% milk before being incubated with ab124964 overnight at 4°C. Antibody binding was detected using an anti-rabbit antibody conjugated to HRP, and visualised using ECL development solution [ab133406](#).

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

Lane 1:
Human lung lysate at 20 µg

Lane 2:
A431 (human epidermoid carcinoma cell line) cell lysate at 20 µg

Lane 3:
A549 (human lung carcinoma cell line) cell lysate at 20 µg

Lane 4:
NIH/3T3 (mouse embyro fibroblast cell line) cell lysate at 20 µg

Lane 5:
SV40-LT cell lysate at 20 µg

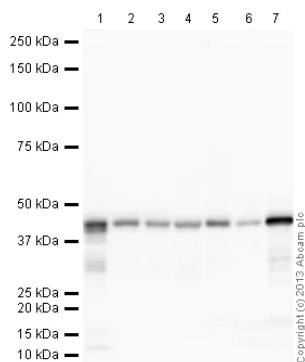
Lane 6:
C2C12 (mouse myoblast cell line) cell lysate at 20 µg

Developed using the ECL technique.

Predicted band size: 42 kDa

Observed band size: 42 kDa

Exposure time: 10s



Western blot - Anti-alpha smooth muscle Actin antibody [EPR5368] (ab124964)

All lanes:
Western blot - Anti-alpha smooth muscle Actin antibody [EPR5368] (ab124964) at 1/1000 dilution

Lane 1:
Human heart tissue lysate - total protein (ab29431) at 10 µg

Lane 2:
Heart (Mouse) Tissue Lysate at 10 µg

Lane 3:
Heart (Rat) Tissue Lysate at 10 µg

Lane 4:
Human skeletal muscle tissue lysate - total protein (ab29330) at 10 µg

Lane 5:
HEK-293 (human epithelial cell line from embryonic kidney) Whole Cell Lysate at 10 µg

Lane 6:
A431 (human epidermoid carcinoma cell line) Whole Cell Lysate at 10 µg

Lane 7:
SV40LT-SMC (rat aorta smooth muscle) Whole Cell Lysate at 10 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/10000 dilution

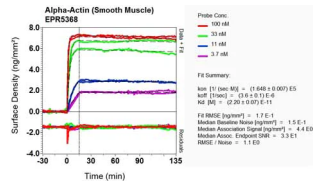
Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 42 kDa

Observed band size: 42 kDa

Exposure time: 30s



OI-RD Scanning - Anti-alpha smooth muscle Actin antibody [EPR5368] (ab124964)

We have systematically measured KD (the equilibrium dissociation constant between the antibody and its antigen), of more than 840 recombinant antibodies to assess not only their individual KD values but also to see the average affinity of antibody. Based on the comparison with published literature values for mouse monoclonal antibodies, Recombinant antibodies appear to be on average 1-2 order of magnitude higher affinity.

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.