abcam

Product datasheet

Anti-alpha smooth muscle Actin antibody [EPR5368] ab124964

KO 評価済 リコンビナント RabMAb

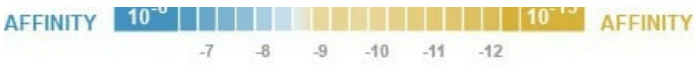
★★★★★ 18 Abreviews 289 References 画像数 26

製品の概要

製品名	Anti-alpha smooth muscle Actin antibody [EPR5368]
製品の詳細	Rabbit monoclonal [EPR5368] to alpha smooth muscle Actin
由来種	Rabbit
アプリケーション	**適用あり:** WB, IHC-P, ICC/IF, Flow Cyt (Intra)
種交差性	**交差種:** Mouse, Rat, Human
免疫原	Synthetic peptide within Human alpha smooth muscle Actin aa 1-100 (N terminal). The exact sequence is proprietary. Database link: **P62736**
ポジティブ・コントロール	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.
特記事項	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**.

製品の特性

製品の状態	Liquid
保存方法	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Avoid freeze / thaw cycle.
解離定数（K_D 値）	$K_D = 2.20 \times 10^{-11}$ M



Learn more about K_D

バッファー	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: 40% Glycerol, 0.05% BSA, PBS
精製度	Protein A purified
ポリ/モノ	モノクローナル
クローン名	EPR5368
アイソタイプ	IgG

アプリケーション

The Abpromise guarantee Abpromise保証は、次のテスト済みアプリケーションにおけるab124964の使用に適用されます

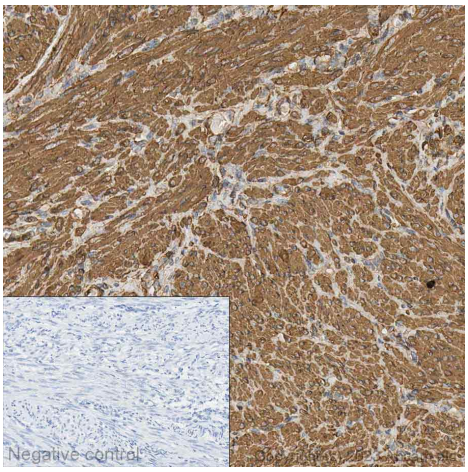
アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
WB	★★★★★ (1)	1/10000 - 1/50000. Detects a band of approximately 42 kDa (predicted molecular weight: 42 kDa). **For unpurified use at 1/1000.**
IHC-P	★★★★★ (10)	1/1000 - 1/2500. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol. See **IHC antigen retrieval protocols**.
ICC/IF	★★★★★ (3)	1/250 - 1/500.
Flow Cyt (Intra)		1/10 - 1/100. **ab172730** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

ターゲット情報

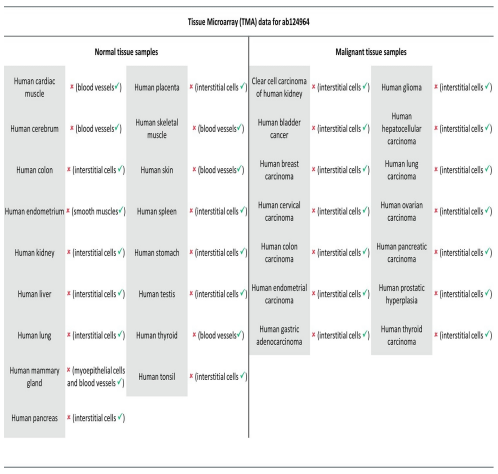
機能	Actins are highly conserved proteins that are involved in various types of cell motility and are ubiquitously expressed in all eukaryotic cells.
関連疾患	Defects in ACTA2 are the cause of aortic aneurysm familial thoracic type 6 (AAT6) [MIM:611788]. AATs are characterized by permanent dilation of the 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.
配列類似性	Belongs to the actin family.
細胞内局在	Cytoplasm > cytoskeleton.

画像



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.



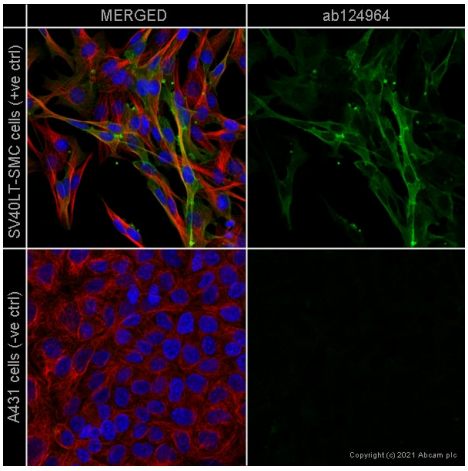
Tissue Microarray (TMA) data for ab124964

Normal tissue samples				Malignant tissue samples			
Human cardiac muscle	✗ (blood vessels ✓)	Human placenta	✗ (interstitial cells ✓)	Clear cell carcinoma of human kidney	✗ (interstitial cells ✓)	Human glioma	✗ (interstitial cells ✓)
Human cerebrum	✗ (blood vessels ✓)	Human skeletal muscle	✗ (blood vessels ✓)	Human bladder cancer	✗ (interstitial cells ✓)	Human hepatocellular carcinoma	✗ (interstitial cells ✓)
Human colon	✗ (interstitial cells ✓)	Human skin	✗ (blood vessels ✓)	Human breast carcinoma	✗ (interstitial cells ✓)	Human lung carcinoma	✗ (interstitial cells ✓)
Human endometrium	✗ (smooth muscles ✓)	Human spleen	✗ (interstitial cells ✓)	Human cervical carcinoma	✗ (interstitial cells ✓)	Human ovarian carcinoma	✗ (interstitial cells ✓)
Human kidney	✗ (interstitial cells ✓)	Human stomach	✗ (interstitial cells ✓)	Human colon carcinoma	✗ (interstitial cells ✓)	Human pancreatic carcinoma	✗ (interstitial cells ✓)
Human liver	✗ (interstitial cells ✓)	Human testis	✗ (interstitial cells ✓)	Human endometrial carcinoma	✗ (interstitial cells ✓)	Human prostatic hyperplasia	✗ (interstitial cells ✓)
Human lung	✗ (interstitial cells ✓)	Human thyroid	✗ (blood vessels ✓)	Human gastric adenocarcinoma	✗ (interstitial cells ✓)	Human thyroid carcinoma	✗ (interstitial cells ✓)
Human mammary gland	✗ (myoepithelial cells and blood vessels ✓)	Human tonsil	✗ (interstitial cells ✓)				
Human pancreas	✗ (interstitial cells ✓)						

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

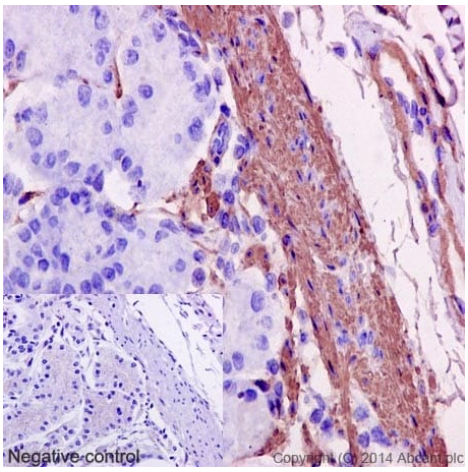
Tissue Microarrays stained for Anti-alpha smooth muscle Actin antibody [EPR5368] using ab124964 in immunohistochemical analysis. This table provides a detailed overview of positive (tick mark) and negaive (cross mark) staining per sample type tested. The section was incubated with ab124964 for 30 mins 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.



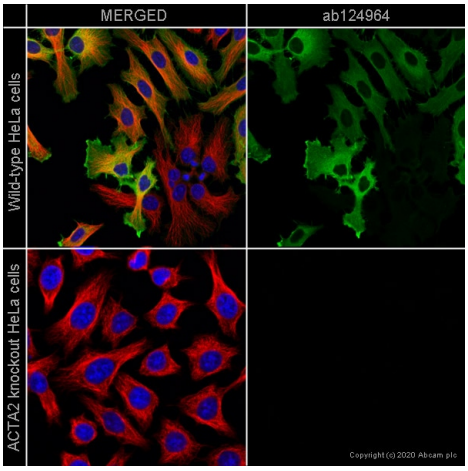
Immunocytochemistry/ Immunofluorescence - Anti-alpha smooth muscle Actin antibody [EPR5368] (ab124964)

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).



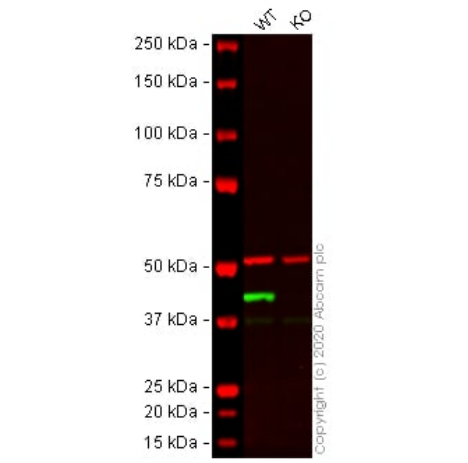
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-alpha smooth muscle Actin antibody [EPR5368] (ab124964)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis 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.



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

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.

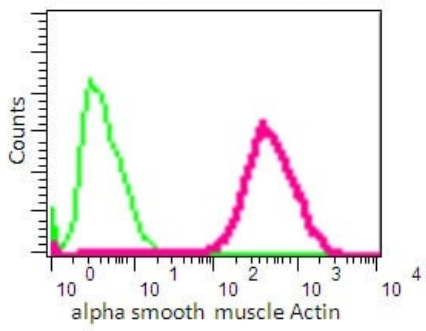
Performed under reducing conditions.

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

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.

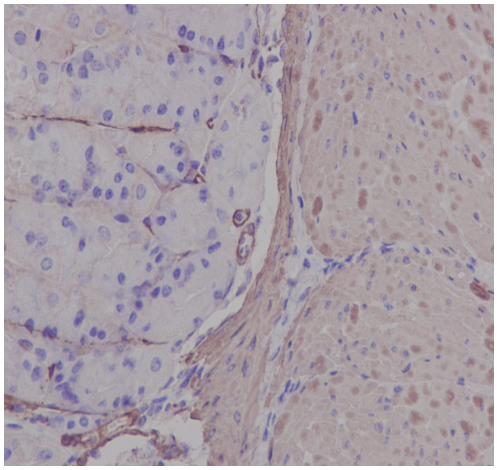
ab124964 was shown to react with alpha smooth muscle Actin in wild-type HeLa cells in western blot. Loss of signal was observed when ACTA2 knockout sample was used. Wild-type HeLa and ACTA2 knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3% milk in TBS-T (0.1% Tween®) before 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] (ab124964)

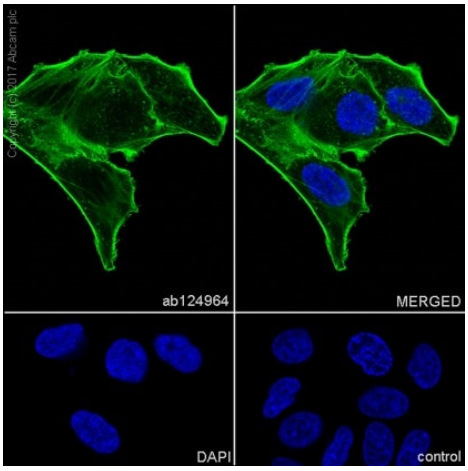
Intracellular Flow Cytometry analysis of Jurkat (human T cell leukemia cell line from peripheral blood) cells labelling alpha smooth muscle Actin with purified ab124964 at 1/30 (red). Cells were fixed with 2% paraformaldehyde. A FITC-conjugated goat anti-rabbit IgG (1/150) was used as the secondary antibody.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



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

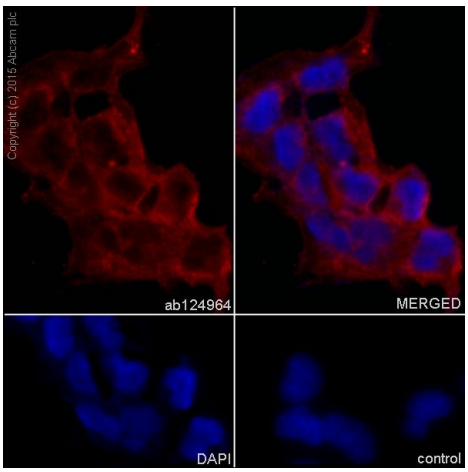
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of mouse stomach tissue labelling alpha smooth muscl Actin with ab124964 at 1/1000. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. A prediluted HRP-polymer conjugated anti-rabbit IgG was used as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

Positive staining on smooth muscle in mouse stomach.



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

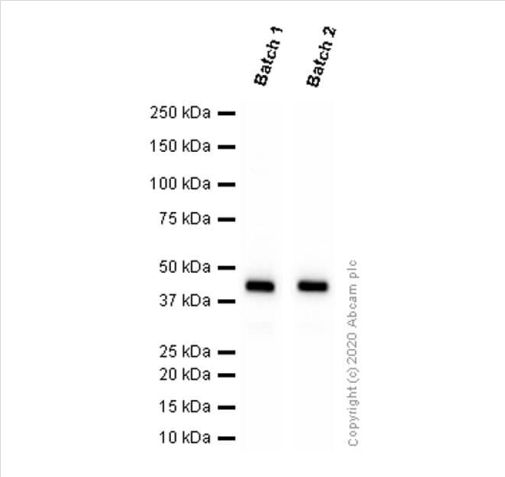
Immunocytochemistry/Immunofluorescence analysis of HeLa cells labeling alpha smooth muscle Actin with ab124964 at 1/500. Cells were fixed with 4% paraformaldehyde and permeabilised with 0.1% tritonX-100. An Goat anti rabbit IgG(Alexa Fluor® 488) **ab150077** (1/1000) was used as the secondary antibody. DAPI (blue) was used as the nuclear counterstain.



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

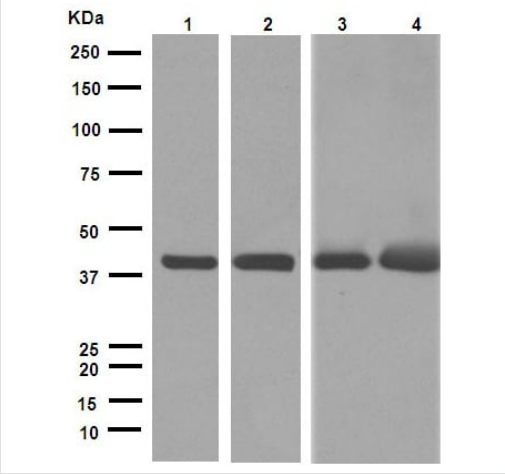
Immunocytochemistry/Immunofluorescence analysis of A-673 (human muscle Ewing's Sarcoma cell line) cells labelling alpha smooth muscle Actin with purified ab124964 at 1/300. Cells were fixed with 4% paraformaldehyde. An Alexa Fluor® 555-conjugated goat anti-rabbit IgG (1/500) was used as the secondary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



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

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.



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

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

Lane 1 : HeLa (human epithelial cell line from cervix adenocarcinoma) cell lysate
Lane 2 : HEK-293 (human epithelial cell line from embryonic kidney) cell lysate
Lane 3 : U937 (human histiocytic lymphoma cell line) cell lysate
Lane 4 : A431 (human epidermoid carcinoma cell line) cell lysate

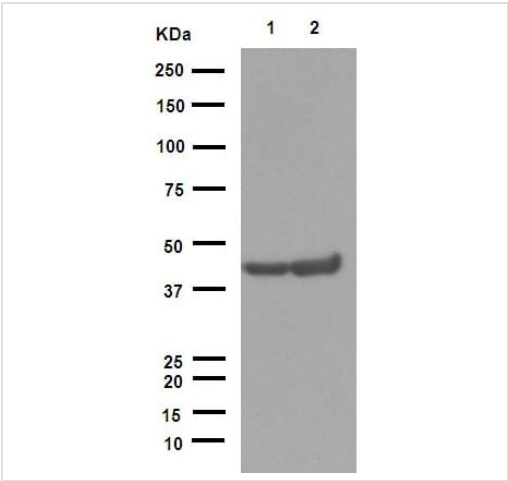
Lysates/proteins at 20 µg per lane.

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

Blocking buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM /TBST.



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

All lanes : Anti-alpha smooth muscle Actin antibody [EPR5368] (ab124964) at 1/50000 dilution (purified)

Lane 1 : Mouse brain tissue lysate
Lane 2 : Rat brain tissue lysate

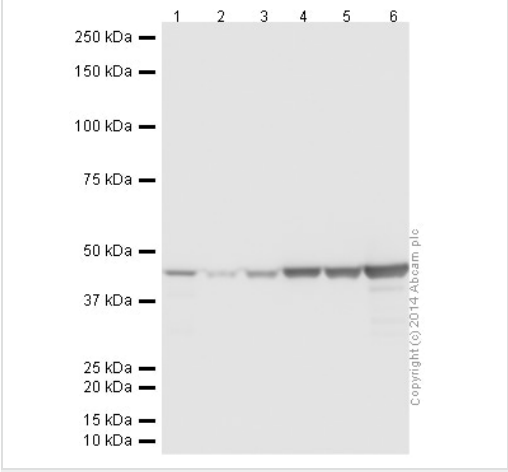
Lysates/proteins at 20 µg per lane.

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

Blocking buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM /TBST.



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

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

Lane 1 : Human lung lysate
Lane 2 : A431 (human epidermoid carcinoma cell line) cell lysate
Lane 3 : A549 (human lung carcinoma cell line) cell lysate
Lane 4 : NIH/3T3 (mouse embyro fibroblast cell line) cell lysate
Lane 5 : SV40-LT cell lysate
Lane 6 : C2C12 (mouse myoblast cell line) cell lysate

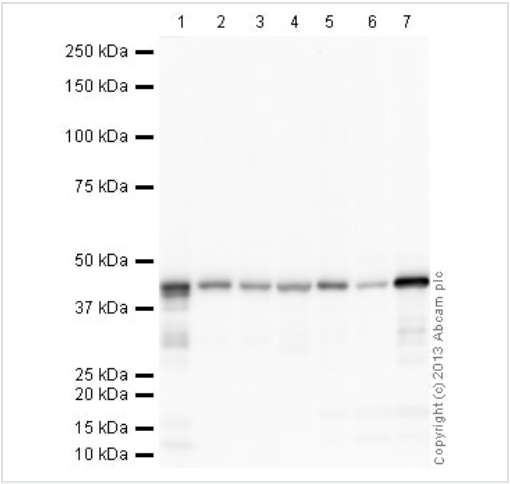
Lysates/proteins at 20 µg per lane.

Developed using the ECL technique.

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

Exposure time: 10 seconds

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**



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

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

Lane 1 : Human heart tissue lysate - total protein (**ab29431**)
Lane 2 : Heart (Mouse) Tissue Lysate
Lane 3 : Heart (Rat) Tissue Lysate
Lane 4 : Human skeletal muscle tissue lysate - total protein (**ab29330**)
Lane 5 : HEK-293 (human epithelial cell line from embryonic kidney) Whole Cell Lysate
Lane 6 : A431 (human epidermoid carcinoma cell line) Whole Cell Lysate
Lane 7 : SV40LT-SMC (rat aorta smooth muscle) Whole Cell Lysate

Lysates/proteins at 10 µg per lane.

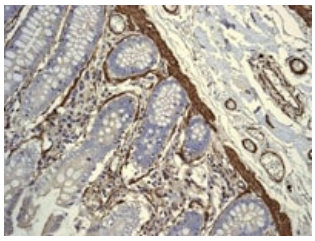
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/10000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 42 kDa
Additional bands at: 42 kDa. We are unsure as to the identity of these extra bands.

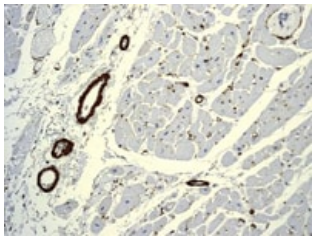
Exposure time: 30 seconds



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

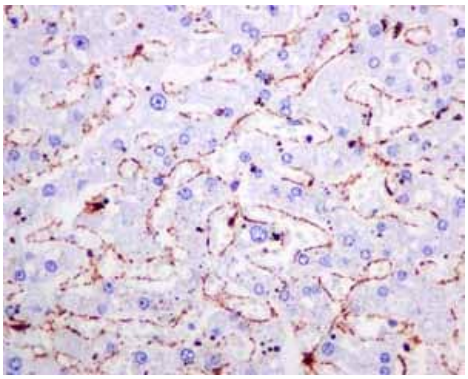
Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.



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

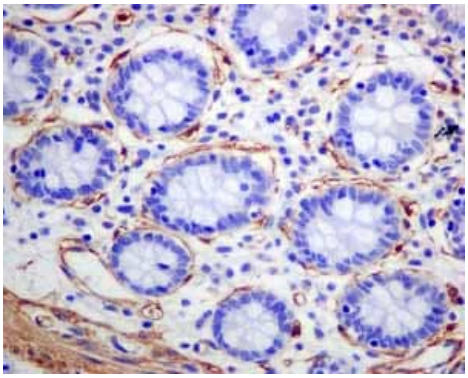
Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.



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

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

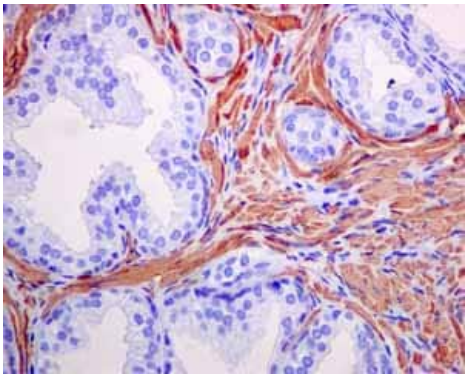
Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.



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

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

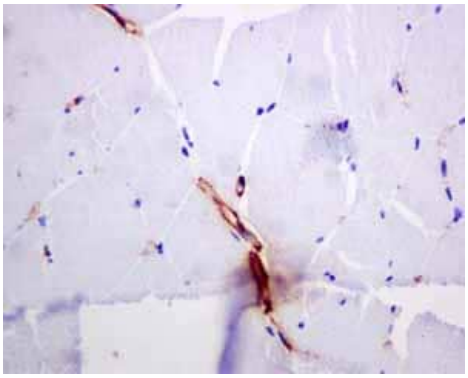
Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.



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

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

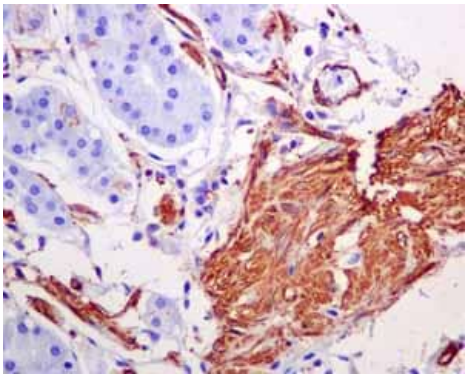
Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.



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.

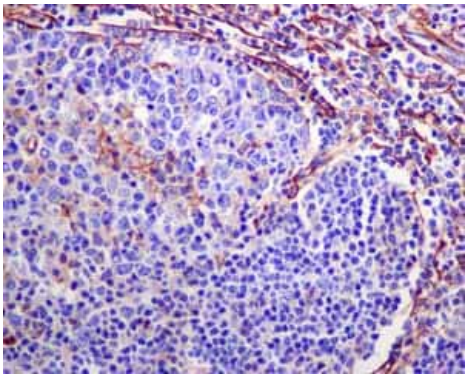
Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.



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

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

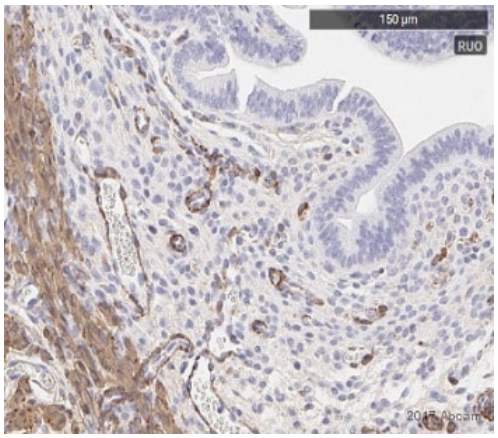
Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.



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

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

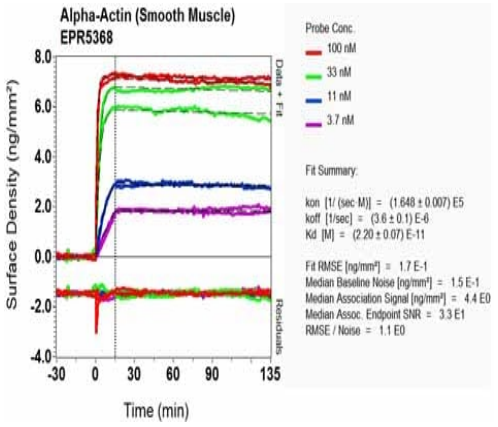
Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.



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

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.

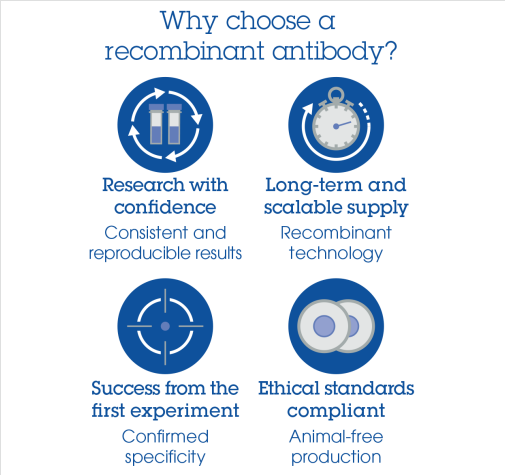


OI-RD Scanning - Anti-alpha smooth muscle Actin antibody [EPR5368] (ab124964)

Equilibrium disassociation constant (K_D)

Learn more about K_D

Click here to learn more about K_D



Anti-alpha smooth muscle Actin antibody

[EPR5368] (ab124964)

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