# abcam

## Product datasheet

# Anti-alpha smooth muscle Actin antibody [EPR5368] ab124964





重组 RabMAb

★★★★★ 18 Abreviews 289 References 26 图像

概述

产品名称 Anti-alpha smooth muscle Actin抗体[EPR5368]

描述 兔单克隆抗体[EPR5368] to alpha smooth muscle Actin

宿主 Rabbit

经测试应用 适用于: WB, IHC-P, ICC/IF, Flow Cyt (Intra)

种属反应性 与反应: 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

阳性对照 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.

常规说明 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**.

性能

形式 Liquid

存放说明 Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C.

Avoid freeze / thaw cycle.

解离常数(KD)  $K_D = 2.20 \times 10^{-11} M$ 

10<sup>-11</sup>

### Learn more about K<sub>D</sub>

**存储溶液** pH: 7.2

Preservative: 0.01% Sodium azide

Constituents: 40% Glycerol, 0.05% BSA, PBS

纯**度** Protein A purified

**克隆** 单克隆

**克隆编号** EPR5368

**同种型** IgG

#### 应用

#### The Abpromise guarantee Abpromise™承诺保证使用ab124964于以下的经测试应用

"应用说明"部分下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
WB	**** <u>(1)</u>	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	<b>★★★★★ (3)</b>	1/250 - 1/500.
Flow Cyt (Intra)		1/10 - 1/100.  ab172730 - Rabbit monoclonal lgG, is suitable for use as an isotype control with this antibody.

#### 靶标

功能 Actins are highly conserved proteins that are involved in various types of cell motility and are

ubiquitously expressed in all eukaryotic cells.

疾病相关 Defects in ACTA2 are the cause of aortic aneurysm familial thoracic type 6 (AAT6) [MIM:611788].

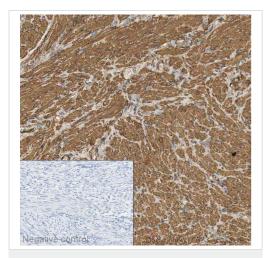
AATs are characterized by permanent dilation of the thoracic aorta usually due to degenerative changes in the aortic wall. They are primarily associated with a characteristic histologic appearance known as 'medial necrosis' or 'Erdheim cystic medial necrosis' in which there is degeneration and fragmentation of elastic fibers, loss of smooth muscle cells, and an

accumulation of basophilic ground substance.

**序列相似性** Belongs to the actin family.

细胞定位 Cytoplasm > cytoskeleton.

#### 图片



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded 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.

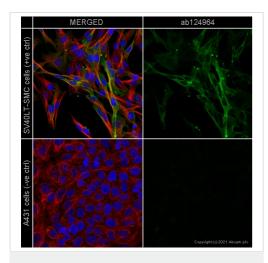
Tissue Microarray (TMA) data for a b124964									
Normal tissue samples				Malignant tissue samples					
Human cardiac muscle	× (blood vessels√)	Human placenta	× (interstitial cells ✓)	Clear cell carcinoma of human kidney	× (interstitial cells √)	Human glioma	<b>×</b> (interstitial cells ✓		
Human 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	<b>≭</b> (interstitial cells ✓)		
uman endometrium	≠ (smooth muscles√)	Human spleen	× (interstitial cells ✓)	Human cervical carcinoma	× (interstitial cells √)	Human ovarian carcinoma			
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	<b>≭</b> (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	x (interstitial cells √)								

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

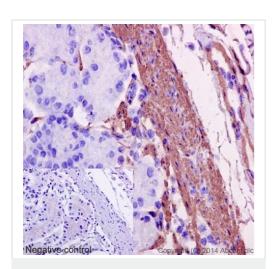
Tissue Microarrays stained for Anti-alpha smooth muscle Actin antibody [EPR5368] using ab124964 in immunohistochemical analysis. This table provides a detailed overview of positive (tick mark) and negaive (cross mark) staining per sample type tested. The section was incubated with ab124964 for 30 mins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



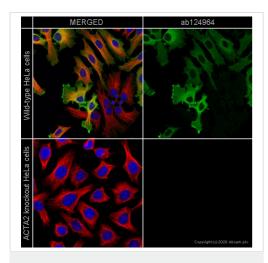
Immunocytochemistry/ Immunofluorescence - Antialpha smooth muscle Actin antibody [EPR5368] (ab124964)



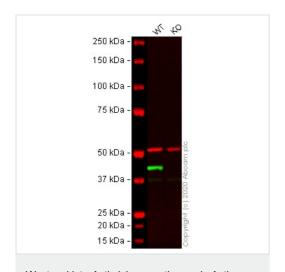
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-alpha smooth muscle
Actin antibody [EPR5368] (ab124964)

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 0.5 $\mu$ g/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 lgG (Alexa Fluor<sup>®</sup> 488) (**ab150081**) at 2  $\mu$ g/ml (shown in green) and a goat secondary antibody to mouse lgG (Alexa Fluor<sup>®</sup> 594) (**ab150120**) at 2  $\mu$ g/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) 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 lgG was used as the secondary antibody. Negative control using PBS instead of primary antibody. Counterstained with Hematoxylin.



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



Western blot - 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  $\underline{ab7291}$  (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 lgG (Alexa Fluor 488) ( $\underline{ab150081}$ ) at 2  $\mu$ g/ml (shown in green) and a goat secondary antibody to mouse lgG (Alexa Fluor 594) ( $\underline{ab150120}$ ) at 2  $\mu$ g/ml (shown in red). Nuclear DNA was labelled in blue with DAPI.

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

**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, <u>ab7291</u> (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<sup>®</sup>) before incubation with ab124964 and <a href="mailto:ab7291">ab7291</a> (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-

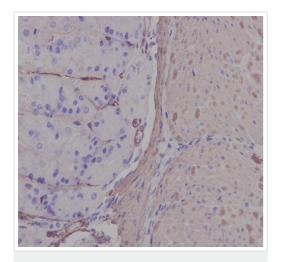
Rabbit IgG H&L (IRDye<sup>®</sup> 800CW) preabsorbed (<u>ab216773</u>) and Goat anti-Mouse IgG H&L (IRDye<sup>®</sup> 680RD) preabsorbed (<u>ab216776</u>) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.

Oonuts

10 10 10 10 10 10 10 alpha smooth muscle Actin

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

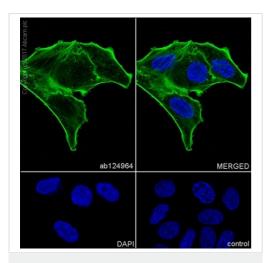
Intracellular Flow Cytometry analysis of Jurkat (human T cell leukemia cell line from peripheral blood) cells labelling alpha smooth muscle Actin with purified ab124964 at 1/30 (red). Cells were fixed with 2% paraformaldehyde. A FITC-conjugated goat antirabbit lgG (1/150) was used as the secondary antibody.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



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded 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 lgG was used as the secondary antibody. Counterstained with Hematoxylin.

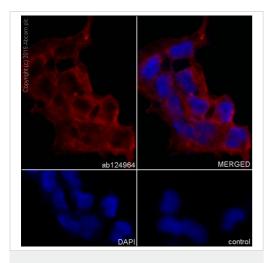
Positive staining on smooth muscle in mouse stomach.



Immunocytochemistry/ Immunofluorescence - Antialpha 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 lgG(Alexa Fluor® 488)

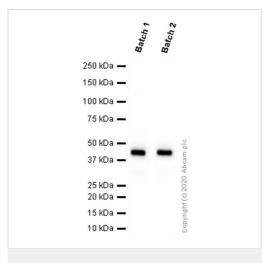
ab150077 (1/1000) was used as the secondary antibody. DAPI (blue) was used as the nuclear counterstain.



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

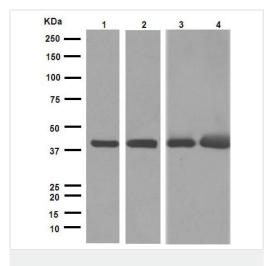
Immunocytochemistry/Immunofluorescence analysis of A-673 (human muscle Ewing's Sarcoma cell line) cells labelling alpha smooth muscle Actin with purified ab124964 at 1/300. Cells were fixed with 4% paraformaldehyde. An Alexa Fluor® 555-conjugated goat anti-rabbit lgG (1/500) was used as the secondary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

Control: primary antibody (1/300) and secondary antibody, <u>**ab150113**</u>, an Alexa Fluor<sup>®</sup> 488-conjugated goat anti-mouse lgG (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  $\mu$ g/ml. 15  $\mu$ g 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.

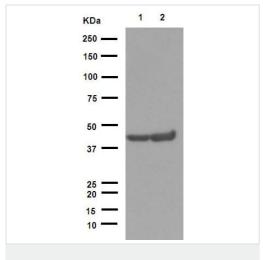
#### **Secondary**

**All lanes :** Peroxidase-conjugated goat anti-rabbit lgG (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 lgG (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.

250 kDa -150 kDa -100 kDa -75 kDa 🕳 50 kDa 🕳 37 kDa 🕳 25 kDa — 20 kDa — 15 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

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** 

1 2 3 4 5 6 7

250 KDa —

150 KDa —

100 KDa —

75 KDa —

37 KDa —

25 KDa —

20 KDa —

10 KDa —

10 KDa —

10 KDa —

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 lgG H&L (HRP) (<u>ab97051</u>) at 1/10000 dilution

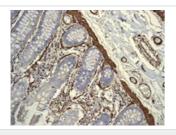
Developed using the ECL technique.

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.

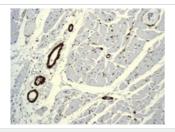
Exposure time: 30 seconds



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded 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.

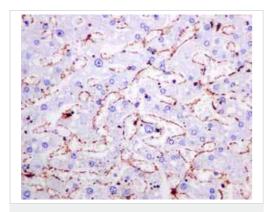
Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded 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.

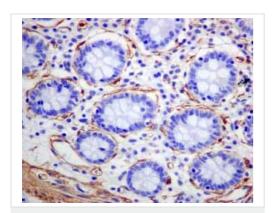
Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded 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.

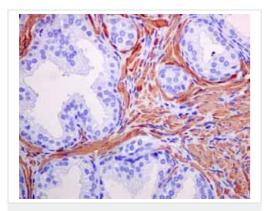
Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded 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.

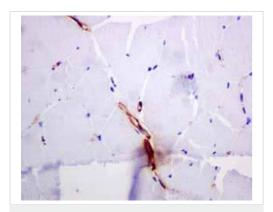
Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded 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.

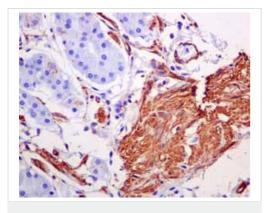
Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded 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.

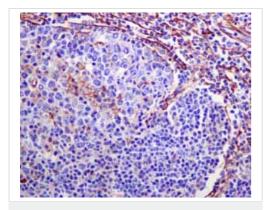
Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded 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.

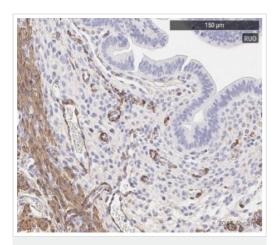
Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded 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.

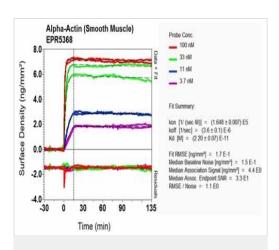
Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded 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 <u>ab64226</u> 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.



Ol-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 KD



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

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