abcam

Product datasheet

Anti-alpha smooth muscle Actin antibody ab5694

**** 150 Abreviews 2625 References 7 Images

Overview

Product name Anti-alpha smooth muscle Actin antibody

Description Rabbit polyclonal to alpha smooth muscle Actin

Host species Rabbit

Specificity Alpha smooth muscle actin antibody (ab5694) stains smooth muscle cells in vessel walls, gut wall,

and myometrium. Myoepithelial cells in breast and salivary gland are also stained. ab5694 reacts

with tumors arising from smooth muscles and myoepithelial cells.

Tested applications Suitable for: WB, IHC-P

Species reactivity Reacts with: Mouse, Human

Immunogen Synthetic peptide corresponding to Human alpha smooth muscle Actin (N terminal).

Database link: P62736

Positive control WB: HEK-293, A431, HeLa, Jurkat and NIH/3T3 whole cell lysate. Mouse heart tissue

homogenate. HeLa nuclear lysate. IHC-P: PACT-sRIMS-cleared virgin and lactating mammary glands. Mouse intestine and pancreas tissue. Mouse aortic regurgitation. Mouse tonsil tissue.

General notes Actins are highly conserved proteins expressed in all eucarvotic cells. Actin filaments form part

Actins are highly conserved proteins expressed in all eucaryotic cells. Actin filaments form part of the cytoskeleton and play essential roles in regulating cell shape and movement. Six distinct actin

isotypes have been identified in mammalian cells. Each is encoded by a separated gene and is

expressed in a developmentally regulated and tissue-specific manner, alpha and beta cytoplasmic actins are expressed in a wide variety of cells; whereas, expression of alpha skeletal,

alpha cardiac, alpha vascular and gamma enteric actins are more restricted to specialized

muscle cell type. Smooth muscle alpha actin is of further interest because it is one of a few genes

whose expression is relatively restricted to vascular smooth muscle cells. Further more,

expression of smooth muscle alpha actin is regulated by hormones, cell proliferation and altered

by pathological conditions including oncogenic transformation and atherosclerosis.

Abcam is committed to meeting high standards of manufacturing and has decided to discontinue this product once the stock runs out as we are unable to secure its future high-quality supply. We suggest <u>ab124964</u>, <u>ab150301</u> or <u>ab7817</u> as possible replacements. We are sorry for any

inconvenience this may cause.

The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets

your needs before purchasing.

If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be

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Properties

Form Liquid

Storage instructions Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long

term. Avoid freeze / thaw cycle.

Storage buffer pH: 7.40

Preservative: 0.02% Sodium azide

Constituent: PBS

Purity Immunogen affinity purified

Clonality Polyclonal

Isotype IgG

Applications

The Abpromise guarantee Our Abpromise guarantee covers the use of ab5694 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
WB	★★★★☆ (28)	Use a concentration of 0.5 - 2 µg/ml. Predicted molecular weight: 42 kDa.
IHC-P	★★★★★ (69)	1/50 - 1/200. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.

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].

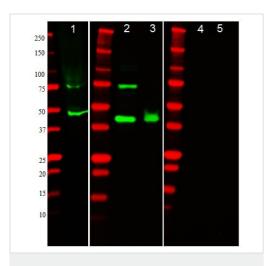
AATs are characterized by permanent dilation of the thoracic aorta usually due to degenerative changes in the aortic wall. They are primarily associated with a characteristic histologic appearance known as 'medial necrosis' or 'Erdheim cystic medial necrosis' in which there is degeneration and fragmentation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



Western blot - Anti-alpha smooth muscle Actin antibody (ab5694)

Lanes 1-3: Anti-alpha smooth muscle Actin antibody (ab5694) at 1 µg/ml

Lane 1 : HEK-293 (Human epithelial cell line from embryonic

kidney) cell lysate - overexpressing alpha-Actin

Lane 2: NIH/3T3 (Mouse embryonic fibroblast cell line) cell lysate

Lanes 3 & 5: Mouse heart tissue homogenate

Lane 4: NIH/3T3 cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Fluor 750-conjugated goat anti-rabbit lgG (H+L) at 1/12500 dilution

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

Incubated with the primary antibody at 4°C overnight.

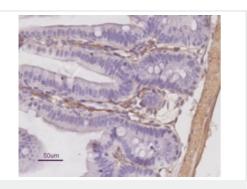
Incubated with the secondary antibody at room temperature for 1 hour.

Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-alpha smooth muscle
Actin antibody (ab5694)

Image from Lloyd-Lewis B. et al., Breast Cancer Res. 2016 18: 127. Fig 2.; doi: 10.1186/s13058-016-0754-9. Reproduced under the Creative Commons license http://creativecommons.org/licenses/by/4.0/.

Passive CLARITY technique (PACT)-sorbitol refractive index matching solution (SRIMS) clearing and 3D imaging of virgin and lactating mouse mammary tissue. **a** PACT-sRIMS tissue clearing and immunostaining protocol and timeline. Three-dimensional confocal imaging of PACT-sRIMS-cleared virgin (**b**) and lactating (**c**) mammary glands immunostained with basal cell markers (K5 and smooth muscle actin (stained with ab5694)) and luminal cell markers (K8 and E-cadherin (E-CAD)). Main image shows the maximum intensity projection of the entire image sequence, with thin optical slices (1 μ m) and their depth (z value) relative to the first image in the image sequence.

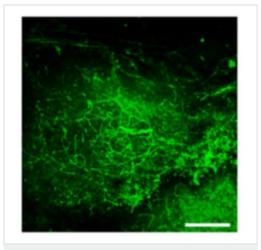
From a paper comparing imaging of intact virgin and lactating mammary glands using 3D imaging of solvent-cleared organs, see deep brain (seeDB), clear unobstructed brain imaging cocktails (CUBIC) and passive clarity technique.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-alpha smooth muscle
Actin antibody (ab5694)

This picture shows formalin-fixed, paraffin embedded mouse intestine and mesentery, the optimal dilution is 1:1600 to 1:3200, incubation overnight at 4°C, counterstained with Hematoxylin.

This image was kindly supplied as part of the review by JQ Zhang.

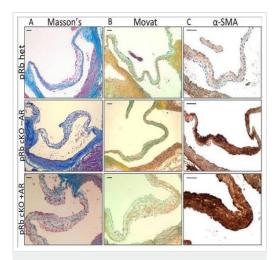


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-alpha smooth muscle
Actin antibody (ab5694)

Image from Lee H. et al., BMC Dev Biol. 2014 Dec 21;14:48. Fig 2Bdoi: 10.1186/s12861-014-0048-3 Reproduced under the Creative Commons license http://creativecommons.org/licenses/by/4.0/.

Pancreatic vessel imaging in the intact adult mouse pancreas. In adult mouse tissues (12 weeks old), imaging was performed after CLARITY. Three-dimensional projection clarified mouse pancreas with capillary immunostained for α -smooth muscle actin (green). Scale bar, 200 μ m.

From a study, that aimed to improve the original CLARITY procedure and developed specific CLARITY protocols for various intact organs.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-alpha smooth muscle
Actin antibody (ab5694)

Freytsis, M. et al PLoS One. 2018 Jan 5;13(1):e0190623. doi: 10.1371/journal.pone.0190623. eCollection 2018 Reproduced under the Creative Commons license http://creativecommons.org/licenses/by/4.0/

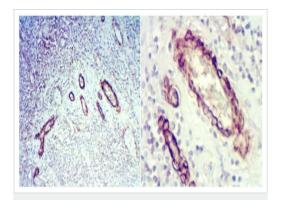
Representative histology of aortic valve leaflets from aged mice demonstrates changes in pRb cKO AoV

A) Masson's trichrome showing reduced collagen staining (blue) in leaflet from pRb cKO mouse with aortic regurgitation (AR). B) Movat pentachrome showing more diffuse collage staining (yellow) in fibrosa, but normal proteoglycan staining (blue) in the spongiosa layer of the leaflet from pRb cKO with AR. **C)**

Immunohistochemistry for α -SMA, demonstrating presence of activated myofibroblasts throughout leaflets of pRb cKO mouse with and without AR. Scale bar is $50\mu m$.

Alpha smooth muscle Actin is detected with ab5694 at 1/1000 dilution.

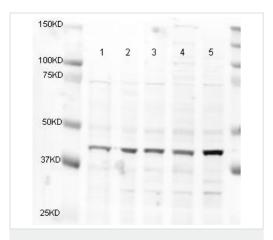
(From Figure 2 of Freytsis et al)



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-alpha smooth muscle
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Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human tonsil tissue labelling alpha smooth muscle Actin with ab5694 at a dilution of 1/1000. Heat mediated antigen retrieval was performed for 35 minutes followed by cooling for 20 minutes. Sections were incubated with the primary antibody for 1 hour followed by incubation with a **biotinylated secondary antibody** for 30 minutes then HRP-Streptavidin for 30 minutes. Developed using DAB chromogen substrate (5-10 minutes). Counter stained with hematoxylin.

Magnification: left - 10X, right - 40X.



Western blot - Anti-alpha smooth muscle Actin antibody (ab5694)

All lanes : Anti-alpha smooth muscle Actin antibody (ab5694) at 1 $\mu g/ml$

Lane 1 : HeLa (Human epithelial cell line from cervix adenocarcinoma) nuclear cell lysate

Lane 2 : HeLa whole cell lysate

Lane 3: A431 (Human epidermoid carcinoma cell line) cell lysate

Lane 4: Jurkat (Human T cell leukemia cell line from peripheral

blood) cell lysate

Lane 5: HEK-293 (Human epithelial cell line from embryonic kidney) cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes: Alexa Fluor anti-rabbit at 1/5000 dilution

Performed under reducing conditions.

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

Additional bands at: 30 kDa, 35 kDa, 37 kDa, 50 kDa, 75 kDa.

We are unsure as to the identity of these extra bands.

Please note that ab5694 does not appear to be specific to smooth muscle.

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