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. The other actins, such as beta and gamma-cytoplasmic, striated muscle and myocardium are not stained by this alpha smooth muscle Actin antibody.

Tested applications
Suitable for: IHC-FoFr, ICC/IF, WB, ELISA, IHC-P, IHC-Fr

Species reactivity
Reacts with: Mouse, Rat, Chicken, Guinea pig, Cow, Dog, Human, Pig

Immunogen
Synthetic peptide corresponding to Human alpha smooth muscle Actin. Alpha smooth muscle actin antibody (ab5694) was raised against a synthetic peptide corresponding to N-terminus of actin from human smooth muscle.

Positive control
WB: HEK-293, NIH/3T3, Hela and jurkat whole cell lysate; Mouse heart tissue lysate; Rat2 myofibroblasts; Pig heart tissue lysate. ICC/IF: Pancreatic cancer cells. Rat myofibroblast cells. Human fetal heart cells IHC-P: Mouse intestine and mesentery tissue. Mouse mammary tissue

General notes
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.

Properties

Form
Liquid

Storage instructions
Storage buffer  
- pH: 7.40
- Preservative: 0.02% Sodium azide
- Constituent: PBS

Purity  
- Immunogen affinity purified

Clonality  
- Polyclonal

Isotype  
- IgG

Applications

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.

<table>
<thead>
<tr>
<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
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</thead>
<tbody>
<tr>
<td>IHC-FoFr</td>
<td>⭐⭐⭐⭐⭐</td>
<td>Use at an assay dependent concentration.</td>
</tr>
<tr>
<td>ICC/IF</td>
<td>⭐⭐⭐⭐⭐</td>
<td>1/100.</td>
</tr>
<tr>
<td>WB</td>
<td>⭐⭐⭐⭐⭐</td>
<td>Use a concentration of 0.5 - 2 µg/ml. Predicted molecular weight: 42 kDa.</td>
</tr>
<tr>
<td>ELISA</td>
<td>⭐⭐⭐⭐⭐</td>
<td>Use a concentration of 0.1 - 1 µg/ml.</td>
</tr>
<tr>
<td>IHC-P</td>
<td>⭐⭐⭐⭐⭐</td>
<td>1/50 - 1/200. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.</td>
</tr>
</tbody>
</table>

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

Lane 1: HEK293 cell lysate - overexpressing alpha-Actin
Lanes 2 & 4: 3T3 cell lysate
Lanes 3 & 5: Mouse heart tissue homogenate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes: Fluor 750-conjugated goat anti-rabbit IgG (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.

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 μm) 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.
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.

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 μm.

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

Ab5694 positively staining smooth muscle cells in blood vessels and myoepithelial cells in the frozen tissue of cancerous human mammary gland (pink) at 1/100 dilution. Secondary; CY5 conjugated goat anti rabbit (1/100). Co immunostaining of glandular cell cytokeratin can be seen stained by FITC (green). Auto fluorescent erythrocytes that are present within blood vessels are shown (red), whilst the DAPI counter stain may clearly be seen staining nuclei (blue).

This image is courtesy of an Abreview submitted by on 22 August 2005. We do not have any further information relating to this image.
Effects of the ROCK1 inhibition on pancreatic cancer cells and cancer-associated fibroblasts

Fluorescence microscopic analysis of fasudil treated, co-cultured pancreatic cancer cells and cancer-associated fibroblasts. Cells were treated with fasudil for 48 hours and then were stained for α-SMA (red), Collagen I (green), and DNA (blue).

Alpha smooth muscle Actin (α-SMA) is detected using ab5694 in 5% formaldehyde-fixed cells.

(From Figure 3C of Watcott et al)

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 collagen 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 50μm.

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

(From Figure 2 of Freytsis et al)
**All lanes**: Anti-alpha smooth muscle Actin antibody (ab5694) at 1/500 dilution

**Lane 1**: Rat2 myofibroblasts (untreated before treatment-0 days)
**Lane 2**: Rat2 myofibroblasts (untreated for 5 days)
**Lane 3**: Rat2 myofibroblasts (treated with 1ng/mL TGF beta)
**Lane 4**: Rat2 myofibroblasts (treated with 10ng/mL TGF beta)
**Lane 5**: Positive control (NIH3T3)
**Lane 6**: Negative control (MDA-MB-469 breast carcinoma cells)

Lysates/proteins at 10 µg per lane.

**Secondary**

**All lanes**: Donkey anti rabbit (HRP) at 1/2500 dilution

Performed under reducing conditions.

**Predicted band size**: 42 kDa

This image is an edited version of an image submitted courtesy of an Abreview on 20 September 2005. We do not have any further information relating to this image.

ab5694 at 1/500 staining rat myofibroblast cells by Immunocytochemistry/ Immunofluorescence. The cells were formaldehyde fixed and blocked with 5% serum prior to incubation with the antibody for 2 hours. A FITC conjugated goat anti-rabbit IgG was used as the secondary. Nuclei were counterstained with propidium iodide.
ab5694 staining Human fetal heart cells by ICC/IF. Cells were fixed with PFA and permeabilized in 0.1% saponin prior to blocking in 10% serum for 45 minutes at 37°C. The primary antibody was diluted 1/400 and incubated with the sample for 1 hour at 37°C. A Alexa Fluor® 594 conjugated goat polyclonal to rabbit IgG (H+L), diluted 1/600 was used as secondary antibody.

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)**

This image is a courtesy of Mario Torrado

**All lanes**: Anti-alpha smooth muscle Actin antibody (ab5694) at 1/1000 dilution

**Lanes 1-2**: Lystates prepared from pig heart tissue from normal control animals

**Lanes 3-4**: Lystates prepared from pig heart tissue from experimental animals

Lysates/proteins at 4 µg per lane.

**Secondary**

**All lanes**: HRP-conjugated goat polyclonal to rabbit IgG at 1/20000 dilution

Performed under reducing conditions.

**Predicted band size**: 42 kDa

**Observed band size**: 45 kDa

*why is the actual band size different from the predicted?*

**Exposure time**: 1 minute

ab5694 staining alpha smooth muscle Actin in rat lung tissue by Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections). Tissue was fixed in formaldehyde and a heat mediated antigen retrieval step was performed using citrate buffer pH 6.0. Samples were then permeabilized using 0.5 Triton X-100 for 20 minutes, blocked with 1% BSA for 30 minutes at 20°C and then incubated with ab5694 at a 1/100 dilution for 16 hours at 4°C. The secondary used was an Alexa-Fluor 568 conjugated goat anti-rabbit polyclonal used at a 1/250 dilution.
Immunohistochemistry (Formalin-fixed paraffin-embedded sections) - Anti-alpha smooth muscle Actin antibody (ab5694)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of skeletal muscle tissue (left) incubated with ab5694 at 1/100 at room temperature for 1 hour showing no specific staining. Right - human tonsil tissue secondary only control.

Heat mediated antigen retrieval was performed for 35 minutes followed by cooling for 20 minutes. A biotinylated secondary antibody was used for 30 minutes followed by incubation with HRP-Streptavidin for 30 minutes. Developed using DAB chromogen substrate (5-10 minutes). Counter stained with hematoxylin. Magnification 10X.

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

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

Lane 1: HeLa Nuclear
Lane 2: HeLa whole cell
Lane 3: A431 cell lysate
Lane 4: Jurkat cell lysate
Lane 5: HEK293 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.
ab5694 staining alpha smooth muscle Actin in human skin tissue section by Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections). Tissue underwent formaldehyde fixation before heat mediated antigen retrieval in Citrate pH 6.0 and then blocked with 10% serum for 1 hour at RT. The primary antibody was diluted 1/300 and incubated with sample in 2% serum for 15 hours at 4°C. A Biotin conjugated goat polyclonal to rabbit IgG was used at dilution at 1/500 as secondary antibody.

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