Overview

Product name: Anti-alpha smooth muscle Actin antibody [1A4] ab7817
Description: Mouse monoclonal [1A4] to alpha smooth muscle Actin
Host species: Mouse
Tested applications: Suitable for: ICC/IF, Flow Cyt (Intra), IHC-P, WB
Species reactivity: Reacts with: Mouse, Rat, Human
Predicted to work with: Sheep, Rabbit, Cow, Pig, Mammals, Baboon
Immunogen: Synthetic peptide corresponding to Human alpha smooth muscle Actin (N terminal).
Database link: P62736
General notes: This antibody clone [1A4] is manufactured by Abcam.
If you require this antibody in a particular buffer formulation or a particular conjugate for your experiments, please contact orders@abcam.com or you can find further information here.

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 found below, along with publications, customer reviews and Q&As.

Properties

Form: Liquid
Storage buffer: pH: 7.40
Preservative: 0.02% Sodium azide
Constituents: PBS, 6.97% L-Arginine

**Purity**
- Protein G purified

**Clonality**
- Monoclonal

**Clone number**
- 1A4

**Isotype**
- IgG2a

**Light chain type**
- kappa

**Applications**

**The Abpromise guarantee**
Our Abpromise guarantee covers the use of ab7817 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>
</tr>
</thead>
<tbody>
<tr>
<td>ICC/IF</td>
<td>★★★★★  (22)</td>
<td>Use a concentration of 1 µg/ml.</td>
</tr>
<tr>
<td>Flow Cyt (Intra)</td>
<td></td>
<td>Use a concentration of 1.137 µg/ml.</td>
</tr>
<tr>
<td>IHC-P</td>
<td>★★★★★  (39)</td>
<td>Use a concentration of 0.034 µg/ml. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.</td>
</tr>
<tr>
<td>WB</td>
<td>★★★★★  (15)</td>
<td>Use a concentration of 1 µg/ml.</td>
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**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**
ab7817 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 ab7817 at 1μg/ml concentration and ab6046 (Rabbit polyclonal to beta 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 mouse IgG (Alexa Fluor® 488) (ab150117) at 2 μg/ml (shown in green) and a goat secondary antibody to rabbit IgG (Alexa Fluor® 594) (ab150080) 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).

**All lanes**: Anti-alpha smooth muscle Actin antibody [1A4] (ab7817) at 1 μg/ml

**Lane 1**: NIH 3T3 whole cell lysate  
**Lane 2**: SV40LT-SMC whole cell lysate  
**Lane 3**: A431 whole cell lysate  
**Lane 4**: A549 whole cell lysate  
**Lane 5**: Jurkat whole cell lysate

Lysates/proteins at 20 μg per lane.

**Secondary**

**All lanes**: Goat anti-Mouse IgG H&L (IRDye® 800RD) at 1/10000 dilution

**Observed band size**: 42 kDa

**Gel type**: MOPS  
**Blocking buffer**: 3% milk  
**Loading control**: alpha tubulin (ab52866), secondary Goat anti-Rabbit IgG H&L (IRDye® 680CW) preadsorbed (1:10000 dilution)
Western blot - Anti-alpha smooth muscle Actin antibody [1A4] (ab7817)

All lanes: Anti-alpha smooth muscle Actin antibody [1A4] (ab7817) at 1 µg/ml

Lane 1: Human colon tissue lysate
Lane 2: Mouse colon tissue lysate
Lane 3: Human Foreskin Fibroblast Whole Cell Lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes: Goat anti-Mouse IgG H&L (IRDye® 800RD) at 1/10000 dilution

Observed band size: 42 kDa

Gel type: MOPS
Blocking buffer: 3% milk
Loading control: alpha tubulin (ab52866), secondary Goat anti-Rabbit IgG H&L (IRDye® 680CW) preadsorbed (1:10000 dilution)

This data was developed using the same antibody clone in a different buffer formulation that is PBS and sodium azide free (ab240654)

ab240654 staining alpha smooth muscle Actin in SV40LT-SMC cells. The cells were fixed with 4% paraformaldehyde (10 min), permeabilized with 0.1% PBS-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 overnight at 4°C with ab240654 at 1µg/ml and ab6046, Rabbit polyclonal to beta Tubulin - Loading Control. Cells were then incubated with ab150117, Goat polyclonal Secondary Antibody to Mouse IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 dilution (shown in green) and ab150080, Goat polyclonal Secondary Antibody to Rabbit IgG - H&L (Alexa Fluor® 594) at 1/1000 dilution (shown in pseudocolour red). Nuclear DNA was labelled with DAPI (shown in blue).

Image was acquired with a high-content analyser (Operetta CLS, Perkin Elmer) and a maximum intensity projection of confocal sections is shown.
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-alpha smooth muscle Actin antibody [1A4] (ab7817)

IHC image of alpha smooth muscle actin staining in a human breast ductal carcinoma formalin fixed paraffin embedded tissue section*, performed on a Leica Bond™ system using the standard protocol F. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab7817, 0.034µg/ml, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX.

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.

*Tissue obtained from the Human Research Tissue Bank, supported by the NIHR Cambridge Biomedical Research Centre

Immunocytochemistry/ Immunofluorescence - Anti-alpha smooth muscle Actin antibody [1A4] (ab7817)

ab7817 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 ab7817 at 5µg/ml concentration and ab6046 (Rabbit polyclonal to beta 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 mouse IgG (Alexa Fluor® 488) (ab150117) at 2 µg/ml (shown in green) and a goat secondary antibody to rabbit IgG (Alexa Fluor® 594) (ab150080) 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).
Flow Cytometry (Intracellular) - Anti-alpha smooth muscle Actin antibody [1A4] (ab7817)

Overlay histogram showing SV40LT-SMC cells stained with ab7817 (red line). The cells were fixed with 4% formaldehyde (10 min) and then permeabilized with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS / 10% normal goat serum to block non-specific protein-protein interactions followed by the antibody (ab7817, 1.137µg/ml) for 30 min at 22°C. The secondary antibody used was Goat Anti-Mouse IgG H&L (Alexa Fluor® 488) (ab150113) at 1/2000 dilution for 30 min at 22°C.

Isotype control antibody (black line) was mouse IgG2a [18C8BC7AD10] (ab170191) used at the same concentration and conditions as the primary antibody. Unlabelled sample (blue line) was also used as a control.

Acquisition of >5,000 events were collected using a 50 mW Blue laser (488nm) and 530/30 bandpass filter.

This antibody gave a positive signal in HeLa cells fixed with 80% methanol (5 min)/permeabilized with 0.1% PBS-Triton X-100 for 15 min used under the same conditions.

Ab7817 staining alpha smooth muscle actin in Mouse intestine tissue by Immunohistochemistry-Immunofluorescence. Tissue was fixed with formaldehyde and blocked with 100% Cas-block for 30 minutes at room temperature; antigen retrieval was performed by heat mediated citrate buffer, pH6. The sample was incubated with primary antibody at 0.034µg/ml for 16 hours at 4°C. An Alexa Fluor® 488 Goat anti-mouse IgG was used as the secondary antibody at 1/400 dilution. Autofluorescence was blocked with 0.1% Sudan Black in 70% ethanol for 10 minutes at room temperature after antigen retrieval, and followed with 3X wash with PBS-T after antigen retrieval. Image was taken with confocal microscope.
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-alpha smooth muscle Actin antibody [1A4] (ab7817)

This image is courtesy of an Abreview submitted by Rudolf Jung

Immunohistochemical analysis of mouse aorta (A) or skin (B) tissue, staining alpha smooth muscle Actin with ab7817.

Tissue was fixed with 10% Neutral Buffered Formalin and blocked with 1% serum for 45 minutes 21°C; antigen retrieval was by enzymatic method in 0.0001% Trypsin-CaCl. Samples were incubated with primary antibody (0.034µg/ml in 0.3% Triton X-100 in PBS) for 1 hour at 21°C. A biotin-conjugated horse anti-mouse polyclonal IgG (1/50) was used as the secondary antibody.
ab7817 staining alpha smooth muscle Actin (green) in Mouse primary colon myofibroblasts by ICC/IF (Immunocytochemistry/Immunofluorescence). Cells were fixed with acetone and blocked with 5% BSA for 30 hours at 25°C. Samples were incubated with primary antibody (1/100 in PBS + 5% BSA) for 2 hours at 25°C. Donkey Anti-Mouse IgG H&L (DyLight® 488) (ab96875) (1/1000) was used as the secondary antibody. Costained with ab92547, Rabbit anti-Vimentin (red).

ab7817 staining alpha smooth muscle Actin in human IMR-90 (Human Lung Fibroblast Cell Line) cells by ICC/IF (Immunocytochemistry/Immunofluorescence). Cells were fixed with paraformaldehyde, permeabilized with 0.1% TritonX-100 and blocked with 100% Cad-Block for 30 minutes at room temperature. Samples were incubated with primary antibody 3.41µg/ml in antibody diluent buffer for 16 hours at 4°C. An Alexa Fluor® 488-conjugated polyclonal Goat anti-mouse IgG, dilution 1/400, was used as secondary antibody.
ab7817 staining alpha smooth muscle Actin in mouse heart cells by ICC/IF (Immunocytochemistry/immunofluorescence). Cells were fixed with paraformaldehyde, permeabilized with TritonX-100 and blocked with 5% BSA for 30 minutes at room temperature. Samples were incubated with primary antibody 6.82µg/ml in blocking buffer for 2 hours. An Alexa Fluor® 488-conjugated Donkey monoclonal to mouse IgG, dilution 1/200, was used as secondary antibody.

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