abcam

Product datasheet

Anti-muscle Actin antibody [EPR8484] ab156302

Recombinant RabMAb

1 Abreviews 8 References 20 Images

Overview

Product name	Anti-muscle Actin antibody [EPR8484]
Description	Rabbit monoclonal [EPR8484] to muscle Actin
Host species	Rabbit
Specificity	ab156302 will detect alpha and gamma specific actin from skeletal, cardiac and smooth muscle.
Tested applications	**Suitable for:** IHC-P, Flow Cyt (Intra), WB, ICC/IF, IP
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide within Human muscle Actin aa 350 to the C-terminus. The exact sequence is proprietary. Database link: **P63267**
Positive control	WB: HeLa, A431, A673, HEK293, MCF7, L6 and NIH 3T3 cell lysates, human fetal artery, kidney and heart tissue lysates, human uterus, stomach and skeletal muscle tissue lysates and rat spleen tissue lysates. IHC-P: Human skeletal muscle, smooth muscle in colon, cervical carcinoma and heart muscle tissues. Mouse cardiac muscle and rat colon tissues. ICC/IF: HeLa, A673 and NIH3T3 cells. Flow Cyt (intra): HeLa cells. IP: NIH 3T3 cell lysates.
General notes	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**.

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. Avoid freeze / thaw cycle.
Storage buffer	Preservative: 0.01% Sodium azide Constituents: 40% Glycerol (glycerin, glycerine), 0.05% BSA, 59% PBS

Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR8484
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab156302 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
IHC-P		1/250. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. See **IHC antigen retrieval protocols**.
Flow Cyt (Intra)		1/10 - 1/100. **ab172730** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
WB	★★☆☆☆ **(1)**	1/1000 - 1/10000. Predicted molecular weight: 42 kDa.
ICC/IF		1/100 - 1/250.
IP		1/10 - 1/100.

Target

Function Actins are highly conserved proteins that are involved in various types of cell motility and are ubiquitously expressed in all eukaryotic cells.

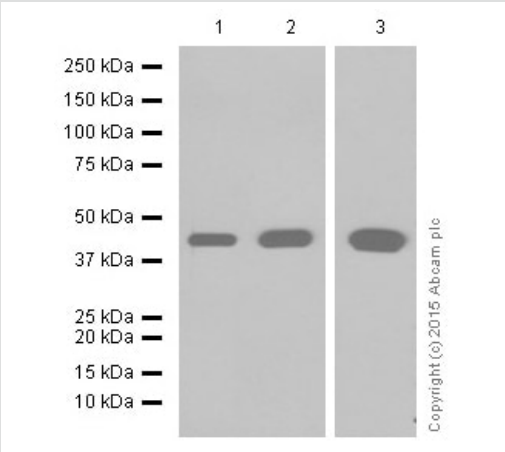
Involvement in disease Defects in ACTA1 are the cause of nemaline myopathy type 3 (NEM3) [MIM:161800]. A form of nemaline myopathy. Nemaline myopathies are muscular disorders characterized by muscle weakness of varying severity and onset, and abnormal thread-or rod-like structures in muscle fibers on histologic examination. The phenotype at histological level is variable. Some patients present areas devoid of oxidative activity containg (cores) within myofibers. Core lesions are unstructured and poorly circumscribed.
Defects in ACTA1 are a cause of myopathy, actin, congenital, with excess of thin myofilaments (MPCETM) [MIM:161800]. A congenital muscular disorder characterized at histological level by areas of sarcoplasm devoid of normal myofibrils and mitochondria, and replaced with dense masses of thin filaments. Central cores, rods, ragged red fibers, and necrosis are absent.
Defects in ACTA1 are a cause of congenital myopathy with fiber-type disproportion (CFTD) [MIM:255310]; also known as congenital fiber-type disproportion myopathy (CFTDM). CFTD is a genetically heterogeneous disorder in which there is relative hypotrophy of type 1 muscle fibers compared to type 2 fibers on skeletal muscle biopsy. However, these findings are not specific and can be found in many different myopathic and neuropathic conditions.

Sequence similarities Belongs to the actin family.

Post-translational Oxidation of Met-46 by MICALs (MICAL1, MICAL2 or MICAL3) to form methionine sulfoxide promotes actin filament depolymerization. Methionine sulfoxide is produced stereospecifically, but

modifications	it is not known whether the (S)-S-oxide or the (R)-S-oxide is produced.
Cellular localization	Cytoplasm > cytoskeleton.

Images



Western blot - Anti-muscle Actin antibody [EPR8484] (ab156302)

All lanes : Anti-muscle Actin antibody [EPR8484] (ab156302) at 1/10000 dilution (purified)

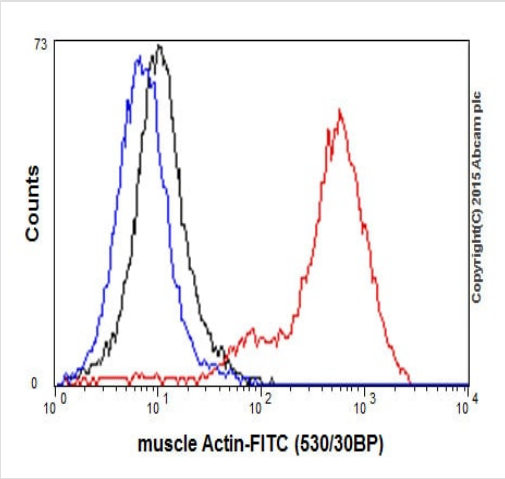
Lane 1 : NIH/3T3 whole cell lysate
Lane 2 : Rat spleen tissue lysate
Lane 3 : L6 whole cell lysate

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : HRP-conjugated goat anti-rabbit IgG (specific to the non-reduced form of IgG) at 1/10000 dilution

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

Blocking and dilution buffer: 5% NFDM /TBST.



Flow Cytometry (Intracellular) - Anti-muscle Actin antibody [EPR8484] (ab156302)

Overlay histogram showing HeLa cells fixed in 4% PFA and stained with purified ab156302 at a dilution of 1 in 50 (red line). The secondary antibody used was FITC goat anti-rabbit at a dilution of 1 in 500. Rabbit monoclonal IgG was used as an isotype control (black line) and cells incubated in the absence of both primary and secondary antibody were used as a negative control (blue line).

Western blot - Anti-muscle Actin antibody [EPR8484] (ab156302)

Anti-muscle Actin antibody [EPR8484] (ab156302) at 1/10000 dilution (purified) + Human stomach tissue lysate at 10 µg

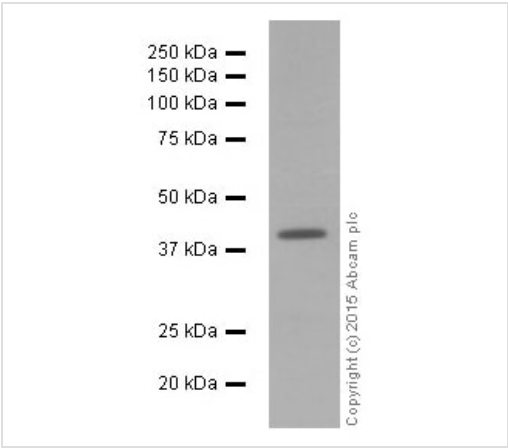
Secondary

HRP-conjugated goat anti-rabbit IgG (specific to the non-reduced form of IgG) at 1/10000 dilution

Predicted band size: 42 kDa

Observed band size: 42 kDa

Blocking and dilution buffer: 5% NFDM /TBST.



Western blot - Anti-muscle Actin antibody [EPR8484] (ab156302)

Anti-muscle Actin antibody [EPR8484] (ab156302) at 1/1000 dilution (purified) + A673 whole cell lysate at 20 µg

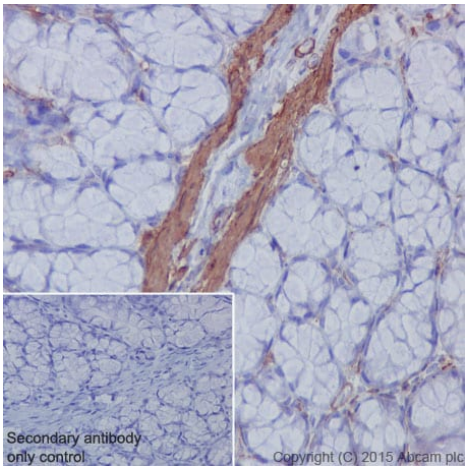
Secondary

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

Predicted band size: 42 kDa

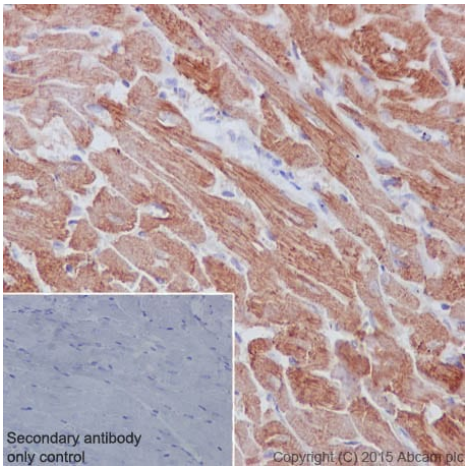
Observed band size: 42 kDa

Blocking and dilution buffer: 5% NFDM /TBST.



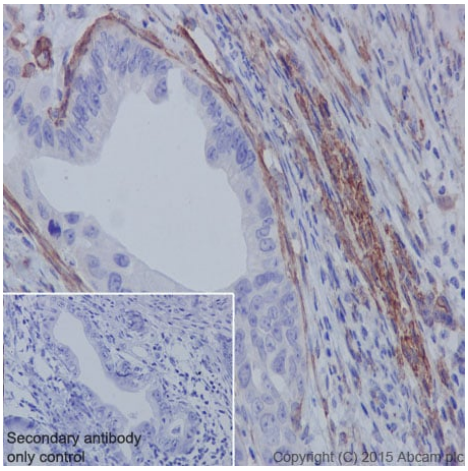
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-muscle Actin antibody [EPR8484] (ab156302)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of rat colon tissue labelling muscle Actin with purified ab156302 at a dilution of 1/250. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. **ab97051**, a HRP-conjugated goat anti-rabbit IgG (H+L) was used as the secondary antibody (1/500). Negative control using PBS instead of primary antibody. Counterstained with hematoxylin.



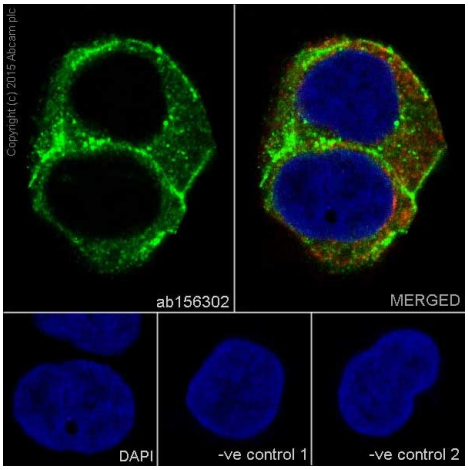
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-muscle Actin antibody [EPR8484] (ab156302)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of mouse cardiac muscle tissue labelling muscle Actin with purified ab156302 at a dilution of 1/250. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. **ab97051**, a HRP-conjugated goat anti-rabbit IgG (H+L) was used as the secondary antibody (1/500). Negative control using PBS instead of primary antibody. Counterstained with hematoxylin.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-muscle Actin antibody [EPR8484] (ab156302)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human cervical carcinoma tissue labelling muscle Actin with purified ab156302 at a dilution of 1/250. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. **ab97051**, a HRP-conjugated goat anti-rabbit IgG (H+L) was used as the secondary antibody (1/500). Negative control using PBS instead of primary antibody. Counterstained with hematoxylin.

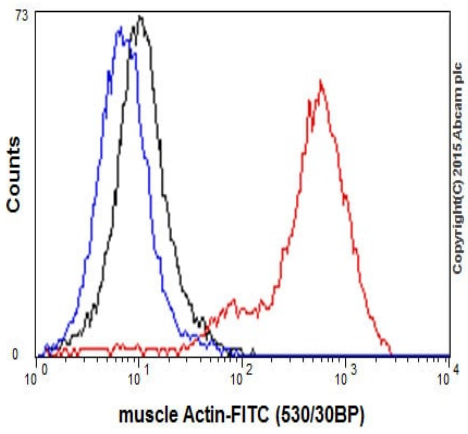


Immunocytochemistry/ Immunofluorescence - Anti-muscle Actin antibody [EPR8484] (ab156302)

Immunocytochemistry/Immunofluorescence analysis of A673 cells labelling muscle Actin with purified ab156302 at 1/100. Cells were fixed with 100% methanol and permeabilized with 0.1% Triton X-100. **ab150077**, an Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/1000) was used as the secondary antibody. DAPI (blue) was used as the nuclear counterstain. **ab7291**, a mouse anti-tubulin (1/1000) and **ab150120**, an Alexa Fluor® 594-conjugated goat anti-mouse IgG (1/1000) were also used.

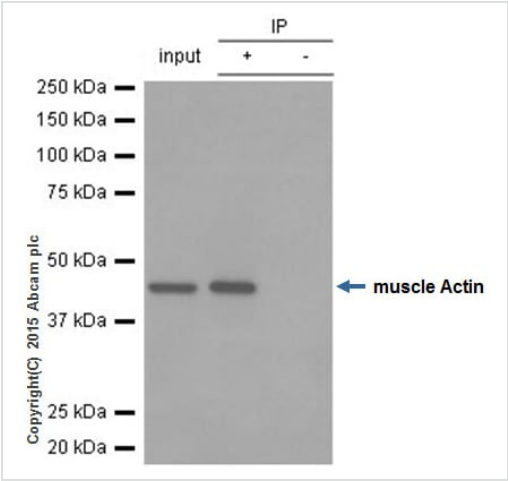
Control 1: primary antibody (1/100) and secondary antibody, **ab150120**, an Alexa Fluor® 594-conjugated goat anti-mouse IgG (1/1000).

Control 2: **ab7291** (1/1000) and secondary antibody, **ab150077**, an Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/1000).



Flow Cytometry (Intracellular) - Anti-muscle Actin antibody [EPR8484] (ab156302)

Intracellular Flow Cytometry analysis ofHeLa cells labelling muscle Actin with purified ab156302 at 1/50 (red). Cells were fixed with 4% paraformaldehyde. An Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/500) was used as the secondary antibody. Black - Isotype control, rabbit monoclonal IgG. Blue - Unlabelled control, cells without incubation with primary and secondary antibodies.



Immunoprecipitation - Anti-muscle Actin antibody [EPR8484] (ab156302)

ab156302 (purified) at 1/20 immunoprecipitating muscle Actin in NIH/3T3 whole cell lysate.

Lane 1 (input): NIH/3T3 whole cell lysate (10µg)

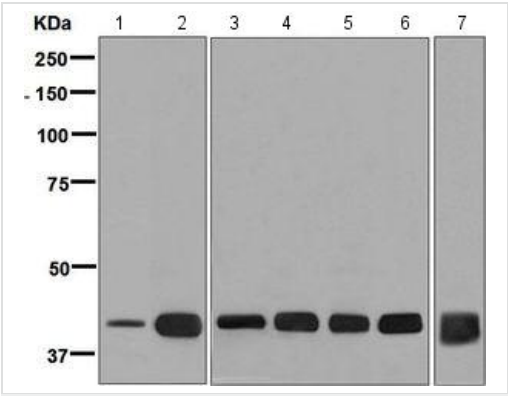
Lane 2 (+): ab156302 + NIH/3T3 whole cell lysate.

Lane 3 (-): Rabbit monoclonal IgG (**ab172730**) instead of ab156302 in NIH/3T3 whole cell lysate.

For western blotting, VeriBlot for IP Detection Reagent (HRP) (**ab131366**), was used for detection at 1/10,000 dilution.

Blocking buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM /TBST.



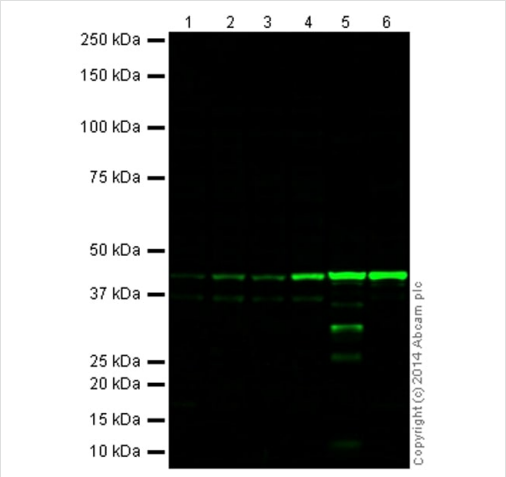
Western blot - Anti-muscle Actin antibody [EPR8484] (ab156302)

All lanes : Anti-muscle Actin antibody [EPR8484] (ab156302) at 1/1000 dilution (unpurified)

Lane 1 : NIH 3T3 lysate
Lane 2 : Human fetal artery lysate
Lane 3 : Human fetal kidney lysate
Lane 4 : Human uterus lysate
Lane 5 : Human stomach lysate
Lane 6 : Human fetal heart lysate
Lane 7 : Human skeletal muscle lysate

Lysates/proteins at 10 µg per lane.

Predicted band size: 42 kDa



Western blot - Anti-muscle Actin antibody [EPR8484] (ab156302)

All lanes : Anti-muscle Actin antibody [EPR8484] (ab156302) at 1/1000 dilution (unpurified)

Lane 1 : A431 (Human epithelial carcinoma cell line) Whole Cell Lysate
Lane 2 : HEK293 (Human embryonic kidney cell line) Whole Cell Lysate
Lane 3 : MCF7 (Human breast adenocarcinoma cell line) Whole Cell Lysate
Lane 4 : HeLa (Human epithelial carcinoma cell line) Whole Cell Lysate
Lane 5 : Skeletal Muscle (Human) Tissue Lysate - adult normal tissue
Lane 6 : NIH 3T3 (Mouse embryonic fibroblast cell line) Whole Cell Lysate

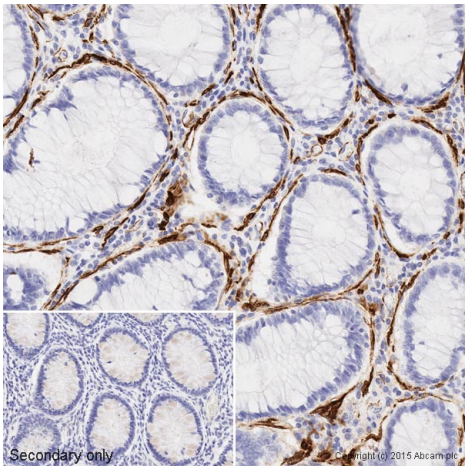
Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (Alexa Fluor® 790) (**ab175781**) at 1/10000 dilution

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

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 Licor blocking buffer before being incubated with ab156302 overnight at 4°C. Antibody binding was detected using **ab175781** at a 1/10,000 dilution for 1hr at room temperature and then imaged using the Licor Odyssey CLx.

Secondary antibody - goat anti-rabbit Alexa Fluor® 790 (**ab175781**).

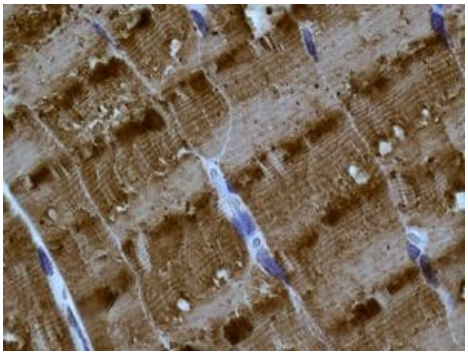


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-muscle Actin antibody [EPR8484] (ab156302)

IHC image of unpurified ab156302 staining muscle Actin in human colon* formalin fixed paraffin embedded tissue sections, performed on a Leica Bond. 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 unpurified ab156302, 5µg/ml working concentration, 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. No primary antibody was used in the secondary only control (shown on the inset).

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.

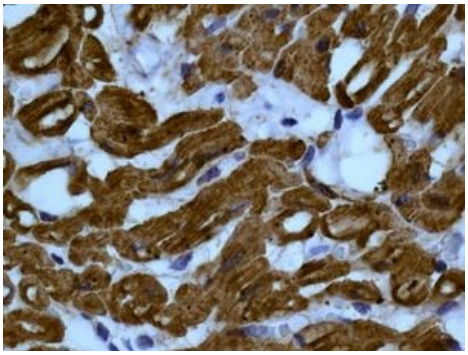
*Tissue obtained from the Human Research Tissue Bank, supported by the NIHR Cambridge Biomedical Research Centre.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-muscle Actin antibody [EPR8484] (ab156302)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human skeletal muscle tissue labelling muscle Actin with unpurified ab156302 at a dilution of 1/1000.

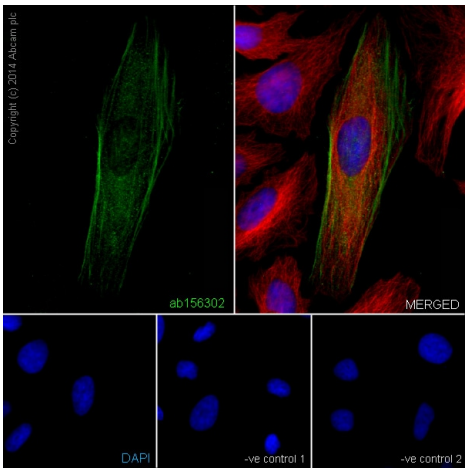
Heat mediated antigen retrieval was performed before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-muscle Actin antibody [EPR8484] (ab156302)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human heart muscle tissue labelling muscle Actin with unpurified ab156302 at a dilution of 1/1000.

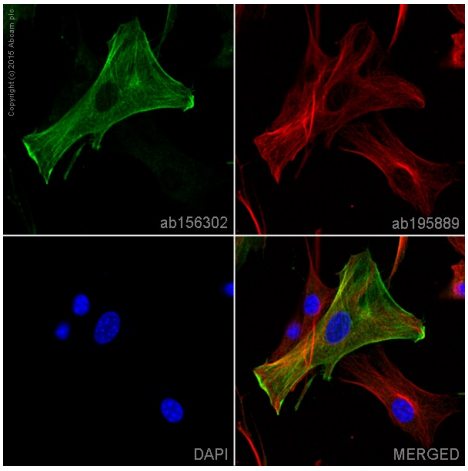
Heat mediated antigen retrieval was performed before commencing with IHC staining protocol.



Immunocytochemistry/ Immunofluorescence - Anti-muscle Actin antibody [EPR8484] (ab156302)

Unpurified ab156302 staining Actin in HeLa cells. The cells were fixed with 100% methanol (5min) and then blocked in 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with unpurified ab156302 at 10µg/ml and **ab7291** at 1µg/ml overnight at +4°C, followed by a further incubation at room temperature for 1h with an goat anti-rabbit Alexa Fluor® 488 secondary (**ab150081**) at 2 µg/ml (shown in green) and goat anti-mouse Alexa Fluor® 594 secondary (**ab150120**) at 2 µg/ml (shown in pseudo color red). Nuclear DNA was labelled in blue with DAPI.

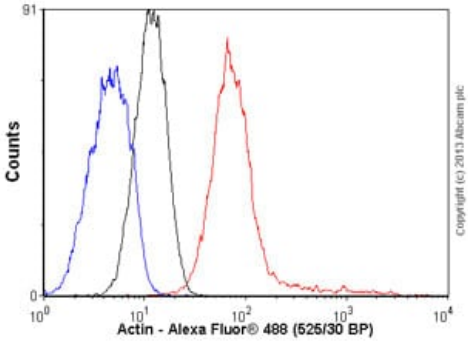
Negative controls: 1 – Rabbit primary and anti-mouse secondary antibody; 2 – Mouse primary antibody and anti-rabbit secondary antibody. Controls 1 and 2 indicate that there is no unspecific reaction between primary and secondary antibodies used.



Immunocytochemistry/ Immunofluorescence - Anti-muscle Actin antibody [EPR8484] (ab156302)

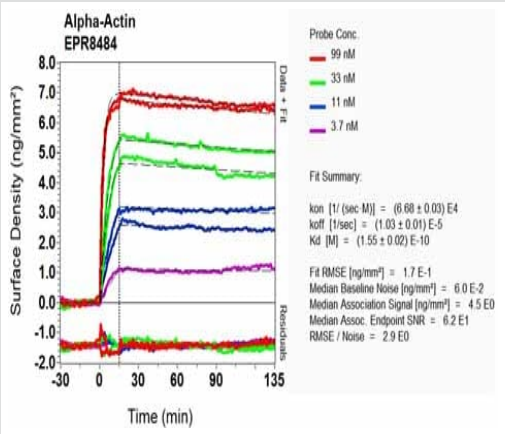
Unpurified ab156302 staining Actin in NIH3T3 cells. The cells were fixed with 100% methanol (5min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked in 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with unpurified ab156302 at 5µg/ml and **ab195889** at 1/250 dilution (shown in pseudo color red) overnight at +4°C, followed by a further incubation at room temperature for 1h with an goat anti-rabbit Alexa Fluor® 488 secondary (**ab150081**) at 2 µg/ml (shown in green). Nuclear DNA was labelled in blue with DAPI.

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



Flow Cytometry (Intracellular) - Anti-muscle Actin antibody [EPR8484] (ab156302)

Overlay histogram showing HeLa cells stained with unpurified ab156302 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Triton X-100 for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (unpurified ab156302, 1/100 dilution) for 30 min at 22°C. The secondary antibody used was goat anti-rabbit Alexa Fluor® 488 (IgG H&L) (**ab150077**) at 1/2000 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) (1µg/1x10^6 cells) used under the same conditions. Unlabelled sample (blue line) was also used as a control. Acquisition of >5,000 events were collected using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter.

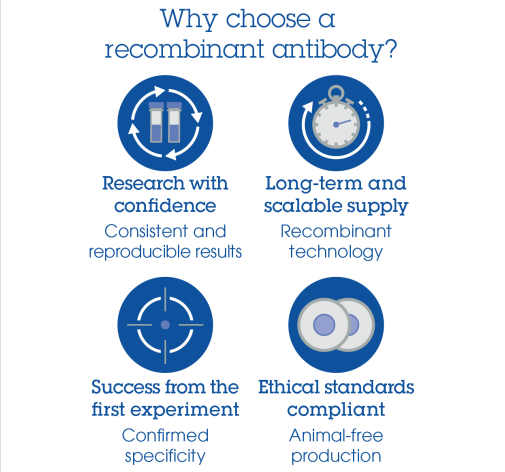


OI-RD Scanning - Anti-muscle Actin antibody [EPR8484] (ab156302)

Equilibrium disassociation constant (K_D)

Learn more about K_D

Click here to learn more about K_D



Anti-muscle Actin antibody [EPR8484] (ab156302)

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours

- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise,

please visit **https://www.abcam.com/abpromise** or contact our technical team.

Terms and conditions

- Guarantee only valid for products bought direct from Abcam or one of our authorized distributors