abcam

Product datasheet

Anti-muscle Actin antibody [EPR8484] ab156302



重组 RabMAb

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概述

产品名称 Anti-muscle Actin抗体[EPR8484]

描述 兔单克隆抗体[EPR8484] to muscle Actin

宿主 Rabbit

特异性 ab156302 will detect alpha and gamma specific actin from skeletal, cardiac and smooth muscle.

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

种属反应性 与反应: Mouse, Rat, Human

免疫原 Synthetic peptide within Human muscle Actin aa 350 to the C-terminus. The exact sequence is

proprietary.

Database link: P63267

阳性对照 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.

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.

存储溶液 Preservative: 0.01% Sodium azide

Constituents: 40% Glycerol (glycerin, glycerine), 0.05% BSA, 59% PBS

纯**度** Protein A purified

克隆 单克隆

克隆编号 EPR8484

同种型 IgG

应用

The Abpromise guarantee Abpromise™承诺保证使用ab156302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
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 lgG, 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.

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 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.

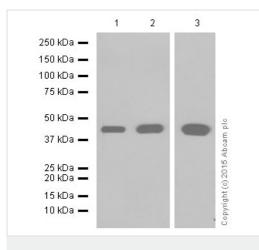
序列相似性

Belongs to the actin family.

翻译后修饰

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

图片



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

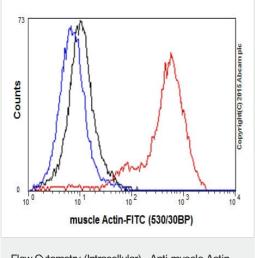
Lysates/proteins at 10 µg per lane.

Secondary

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

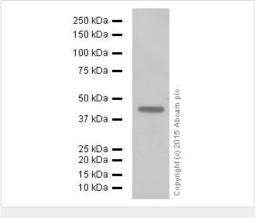
Predicted band size: 42 kDa **Observed band size:** 42 kDa

Blocking and dilution buffer: 5% NFDM /TBST.

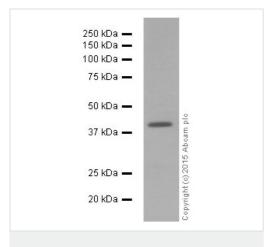


Flow Cytometry (Intracellular) - Anti-muscle Actin antibody [EPR8484] (ab156302)

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)



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 lgG (specific to the non-reduced form of lgG) at 1/10000 dilution

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

Blocking and dilution buffer: 5% NFDM /TBST.

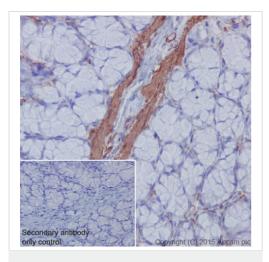
Anti-muscle Actin antibody [EPR8484] (ab156302) at 1/1000 dilution (purified) + A673 whole cell lysate at 20 µg

Secondary

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

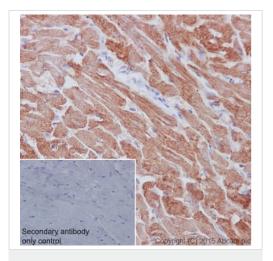
Predicted band size: 42 kDa **Observed band size:** 42 kDa

Blocking and dilution buffer: 5% NFDM /TBST.



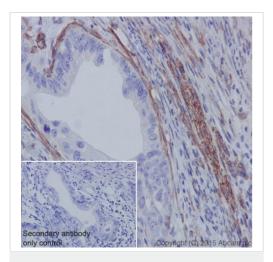
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-muscle Actin antibody
[EPR8484] (ab156302)

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.



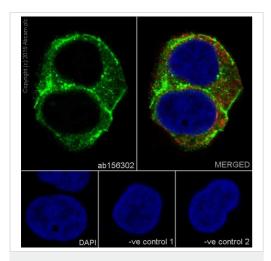
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-muscle Actin antibody [EPR8484] (ab156302)

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 lgG (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 paraffinembedded 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.

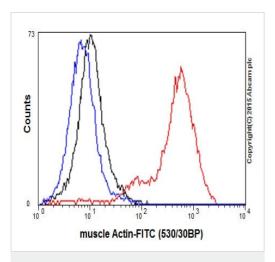


Immunocytochemistry/ Immunofluorescence - Antimuscle Actin antibody [EPR8484] (ab156302)

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 antimouse IgG (1/1000) were also used.

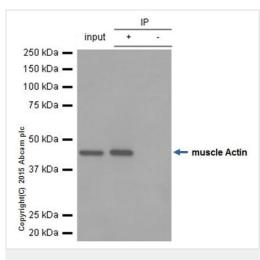
Control 1: primary antibody (1/100) and secondary antibody, **ab150120**, an Alexa Fluor[®] 594-conjugated goat anti-mouse IgG (1/1000).

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

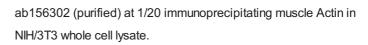


Flow Cytometry (Intracellular) - Anti-muscle Actin antibody [EPR8484] (ab156302)

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)



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

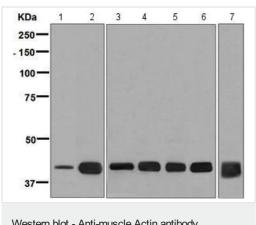
Lane 2 (+): ab156302 + NIH/3T3 whole cell lysate.

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

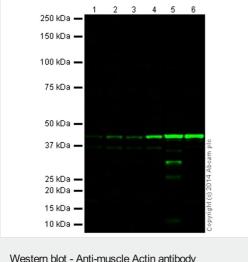
Lane 5: Human stomach lysate

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.

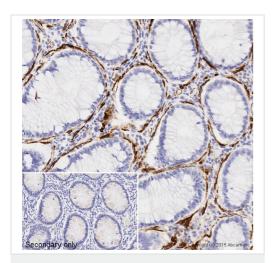
Secondary

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 paraffinembedded sections) - Anti-muscle Actin antibody [EPR8484] (ab156302)

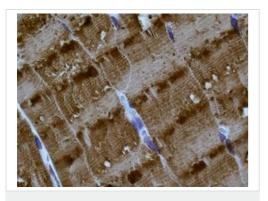
IHC image of unpurified ab156302 staining muscle Actin in human colon* formalin fixed paraffin embedded tissue sections, performed on a Leica Bond. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section 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.

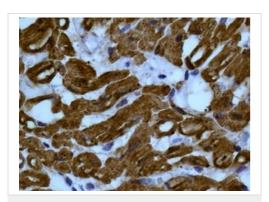
*Tissue obtained from the Human Research Tissue Bank, supported by the NIHR Cambridge Biomedical Research Centre.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) 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.



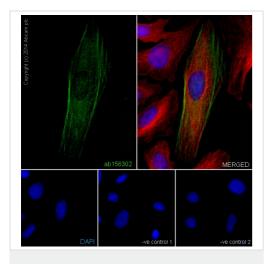
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-muscle Actin antibody [EPR8484] (ab156302)



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded 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.

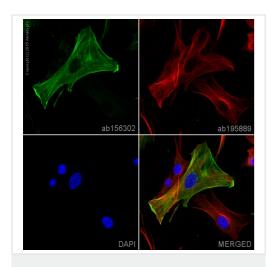
Heat mediated antigen retrieval was performed before commencing with IHC staining protocol.



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

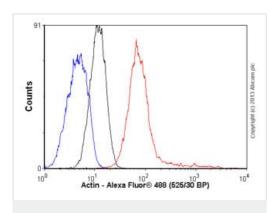
Unpurified ab156302 staining Actin in HeLa cells. The cells were fixed with 100% methanol (5min) and then blocked in 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with unpurified ab156302 at 10µg/ml and ab7291 at 1µg/ml overnight at +4°C, followed by 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.



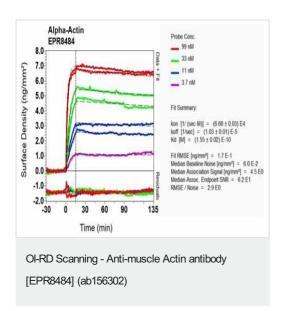
Immunocytochemistry/ Immunofluorescence - Antimuscle Actin antibody [EPR8484] (ab156302)

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 5 μ g/ml and <u>ab195889</u> 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 (<u>ab150081</u>) at 2 μ g/ml (shown in green). Nuclear DNA was labelled in blue with DAPI. Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).



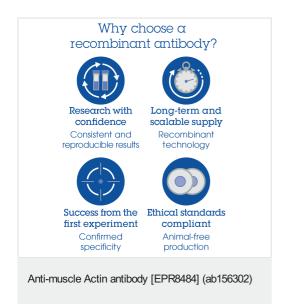
Flow Cytometry (Intracellular) - Anti-muscle Actin antibody [EPR8484] (ab156302)

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 (lgG H&L) (ab150077) at 1/2000 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit lgG (monoclonal) (1µg/1x10⁶ 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.



Equilibrium disassociation constant (K_D) Learn more about K_D

Click here to learn more about K_D



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