abcam

Product datasheet

Anti-alpha Actinin/ACTN1 antibody [EP2527Y] ab68194

敲除验证 重组 RabMAb

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概述

产品名称	Anti-alpha Actinin/ACTN1抗体[EP2527Y]
描述	兔单克隆抗体[EP2527Y] to alpha Actinin/ACTN1
宿主	Rabbit
经测试应用	**适用于:** ICC/IF, IP, WB **不适用于:** IHC-P
种属反应性	**与反应:** Mouse, Rat, Human
免疫原	Synthetic peptide corresponding to Human alpha Actinin/ACTN1.
阳性对照	ICC/IF: ioSkeletal Myocytes - Human iPSC-Derived Skeletal Myocytes (**ab277612**); IP: NIH/3T3 whole cell lysate; WB: HeLa, PC-12, NIH/3T3, and C6 whole 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. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles.
存储溶液	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 0.05% BSA, 40% Glycerol (glycerin, glycerine), 59% PBS
纯度	Protein A purified
克隆	单克隆
克隆编号	EP2527Y
同种型	IgG

应用

The Abpromise guarantee **Abpromise™承诺保证使用ab68194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
ICC/IF		Use a concentration of 0.5 - 5 µg/ml.
IP		1/20.
WB		1/1000. Detects a band of approximately 103 kDa (predicted molecular weight: 103 kDa).

应用说明 Is unsuitable for IHC-P.

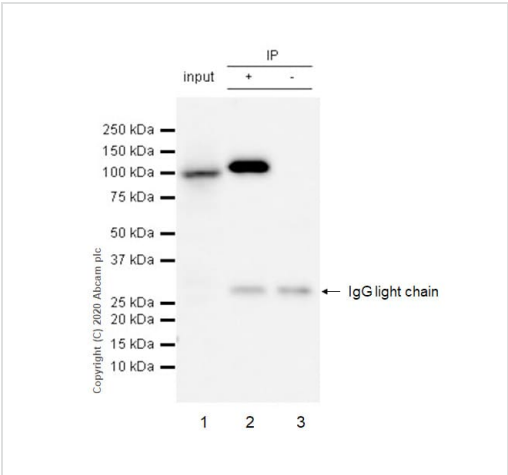
靶标

功能 F-actin cross-linking protein which is thought to anchor actin to a variety of intracellular structures. This is a bundling protein.

序列相似性 Belongs to the alpha-actinin family.
Contains 1 actin-binding domain.
Contains 2 CH (calponin-homology) domains.
Contains 2 EF-hand domains.
Contains 4 spectrin repeats.

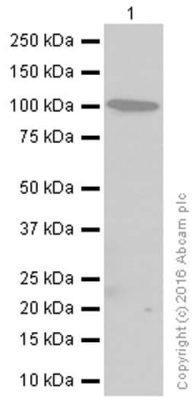
细胞定位 Cytoplasm > cytoskeleton. Cytoplasm > myofibril > sarcomere > Z line. Colocalizes with MYOZ2 and PPP3CA at the Z-line of heart and skeletal muscle.

图片



Immunoprecipitation - Anti-alpha Actinin/ACTN1 antibody [EP2527Y] (ab68194)

Purified ab68194 at 1:20 dilution (2µg) immunoprecipitating alpha Actinin/ACTN1 in NIH/3T3 whole cell lysate.
Lane 1 (input): NIH/3T3 (Mouse embryonic fibroblast) whole cell lysate 10µg
Lane 2 (+): ab68194 + NIH/3T3 whole cell lysate.
Lane 3 (-): Rabbit monoclonal IgG (**ab172730**) instead of ab68194 in NIH/3T3 whole cell lysate.
VeriBlot for IP Detection Reagent (HRP)(**ab131366**) (1:5000 dilution) was used for Western blotting.
Blocking Buffer and concentration: 5% NFDM/TBST.
Diluting buffer and concentration: 5% NFDM/TBST.
Observed band size: 103 kDa



Western blot - Anti-alpha Actinin/ACTN1 antibody [EP2527Y] (ab68194)

Anti-alpha Actinin/ACTN1 antibody [EP2527Y] (ab68194) at 1/10000 dilution (Purified) + HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate at 15 µg

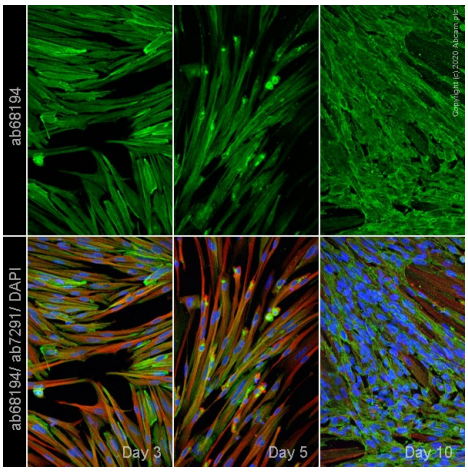
Secondary

Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/50000 dilution

Predicted band size: 103 kDa

Observed band size: 103 kDa

Blocking buffer: 5% NFDM/TBST

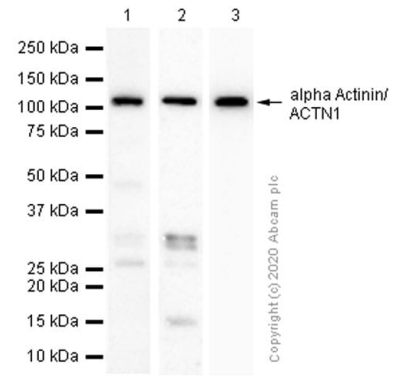


Immunocytochemistry/ Immunofluorescence - Anti-alpha Actinin/ACTN1 antibody [EP2527Y] (ab68194)

Immunofluorescence staining of Actinin/ACTN1 using ab68194 in ioSkeletal Myocytes - Human iPSC-Derived Skeletal Myocytes (**ab277612**), which were differentiated for 3 (left panel), 5 (middle panel) and 10 days (right panel) post induction.

The cells were fixed with 100% MeOH (5 min) 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 ab68194 at 0.5 µg/mL and **ab7291**, Mouse monoclonal [DM1A] to alpha Tubulin, at 1/1000 dilution. Cells were then incubated with **ab150081**, Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 dilution (shown in green) and **ab150120**, Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) preadsorbed at 1/1000 dilution (shown in 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. Gamma is adjusted to 1.5 in all channels.



Western blot - Anti-alpha Actinin/ACTN1 antibody [EP2527Y] (ab68194)

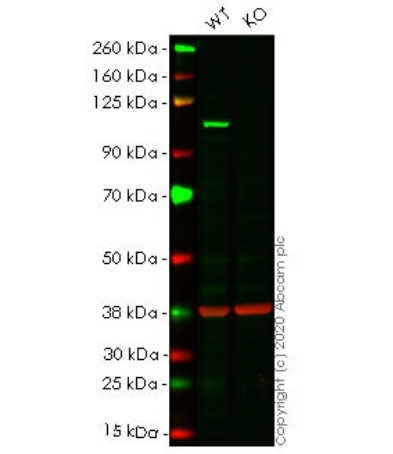
All lanes : Anti-alpha Actinin/ACTN1 antibody [EP2527Y] (ab68194) at 1/1000 dilution (Purified)

Lane 1 : PC-12 (Rat adrenal gland pheochromocytoma) whole cell lysate at 20 µg
Lane 2 : C6 (Rat glial tumor glial cell) whole cell lysate at 15 µg
Lane 3 : NIH/3T3 (Mouse embryonic fibroblast) whole cell lysate at 15 µg

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

Predicted band size: 103 kDa
Observed band size: 103 kDa

Blocking buffer: 5% NFDM/TBST



Western blot - Anti-alpha Actinin/ACTN1 antibody [EP2527Y] (ab68194)

All lanes : Anti-alpha Actinin/ACTN1 antibody [EP2527Y] (ab68194) at 1/2000 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : ACTN1 knockout HeLa cell lysate

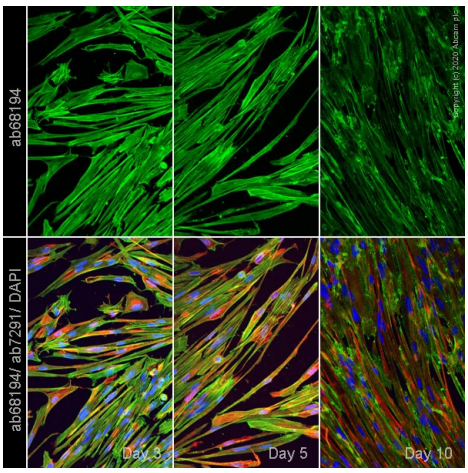
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 103 kDa
Observed band size: 103 kDa

Lanes 1- 2: Merged signal (red and green). Green - ab68194 observed at 103 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) observed at 37 kDa.

ab68194 was shown to react with alpha Actinin/ACTN1 in wild-type HeLa cells in western blot. Loss of signal was observed when knockout cell line **ab265610** (knockout cell lysate **ab257337**) was used. Wild-type HeLa and ACTN1 knockout HeLa cells lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. ab68194 and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated overnight at 4°C at a 1 in 2000 dilution and a 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye®800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye®680RD) preadsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.

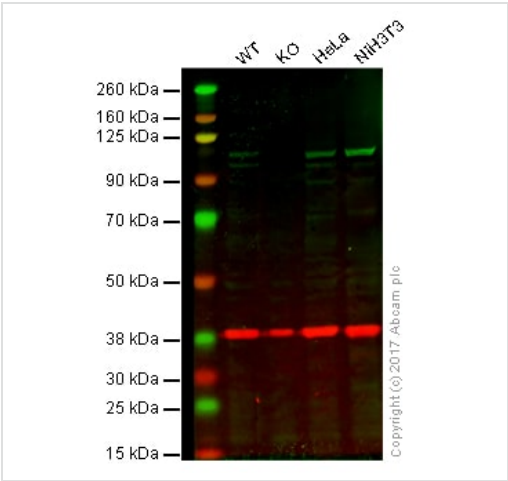


Immunocytochemistry/ Immunofluorescence - Anti-alpha Actinin/ACTN1 antibody [EP2527Y] (ab68194)

Immunofluorescence staining of Actinin/ACTN1 using ab68194 in ioSkeletal Myocytes - Human iPSC-Derived Skeletal Myocytes (**ab277612**), which were differentiated for 3 (left panel), 5 (middle panel) and 10 days (right panel) post induction.

The cells were fixed with 4% formaldehyde (10 min), permeabilized with 0.1% PBS-Tween for 5 mins 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 ab68194 at 0.5 µg/mL and **ab7291**, Mouse monoclonal [DM1A] to alpha Tubulin, at 1/1000 dilution. Cells were then incubated with **ab150081**, Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 dilution (shown in green) and **ab150120**, Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) preadsorbed at 1/1000 dilution (shown in 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. Gamma is adjusted to 1.5 in all channels.



Western blot - Anti-alpha Actinin/ACTN1 antibody [EP2527Y] (ab68194)

Lane 1: Wild-type HAP1 whole cell lysate (20 µg)
Lane 2: Alpha Actinin/ACTN1 knockout HAP1 whole cell lysate (20 µg)
Lane 3: HeLa whole cell lysate (20 µg)
Lane 4: NIH 3T3 whole cell lysate (20 µg)

Lanes 1 - 4: Merged signal (red and green). Green - ab68194 observed at 103 kDa. Red - loading control, **ab9484**, observed at 37 kDa.

ab68194 was shown to specifically react with alpha Actinin/ACTN1 in wild-type HAP1 cells as signal was lost in alpha Actinin/ACTN1 knockout cells. Wild-type and alpha Actinin/ACTN1 knockout samples were subjected to SDS-PAGE. ab68194 and **ab9484** (Mouse anti-GAPDH loading control) were incubated overnight at 4°C at 1/2000 dilution and 1/20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed **ab216773** and Goat anti-Rabbit IgG H&L (IRDye® 680RD) preabsorbed **ab216777** secondary antibodies at 1/20000 dilution for 1 hour at room temperature before imaging.

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