

Anti-alpha Actinin/ACTN1 antibody [EP2527Y] ab68194

KO 評価済 リコンビナント RabMAb

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製品の概要

製品名	Anti-alpha Actinin/ACTN1 antibody [EP2527Y]
製品の詳細	Rabbit monoclonal [EP2527Y] to alpha Actinin/ACTN1
由来種	Rabbit
アプリケーション	適用あり: ICC/IF, IP, WB 適用なし: IHC-P
種交差性	交差種: Mouse, Rat, Human
免疫原	Synthetic peptide corresponding to Human alpha Actinin/ACTN1.
ポジティブ・コントロール	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.
特記事項	<p>This product is a recombinant monoclonal antibody, which offers several advantages including:</p> <ul style="list-style-type: none"> - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production <p>For more information see here.</p> <p>Our RabMAb[®] technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb[®] patents.</p>

製品の特性

製品の状態	Liquid
保存方法	Shipped at 4°C. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles.
バッファー	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 0.05% BSA, 40% Glycerol (glycerin, glycerine), 59% PBS
精製度	Protein A purified
ポリ/モノ	モノクローナル
クローン名	EP2527Y
アイソタイプ	IgG

アプリケーション

The Abpromise guarantee **Abpromise保証は、** 次のテスト済みアプリケーションにおけるab68194の使用に適用されます
アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご確認ください。

アプリケーション	Abreviews	特記事項
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).

追加情報 Is unsuitable for IHC-P.

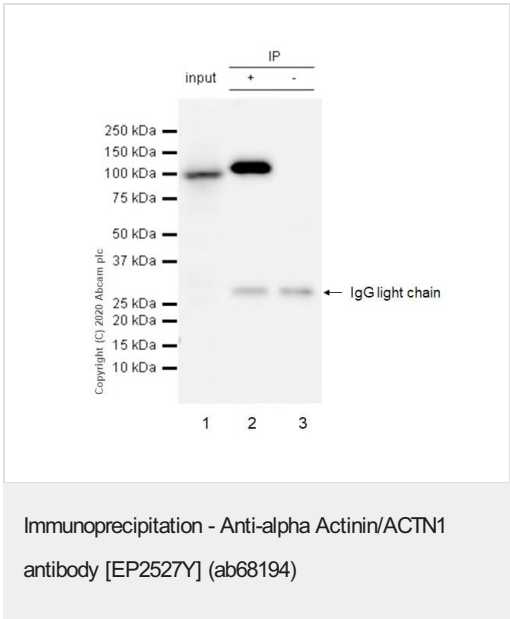
ターゲット情報

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

配列類似性 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.

細胞内局在 Cytoplasm > cytoskeleton. Cytoplasm > myofibril > sarcomere > Z line. Colocalizes with MYO22 and PPP3CA at the Z-line of heart and skeletal muscle.

画像



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.

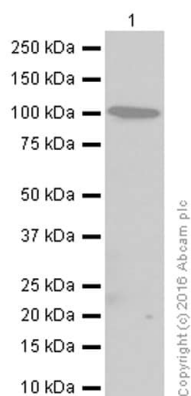
Lane 3 (-): Rabbit monoclonal IgG (**ab172730**) instead of ab68194 in NIH/3T3 whole cell lysate.

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

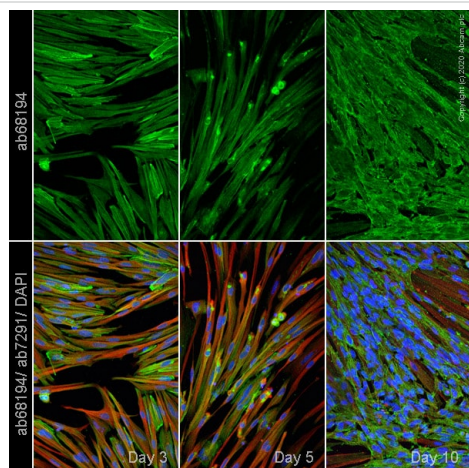
Secondary

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

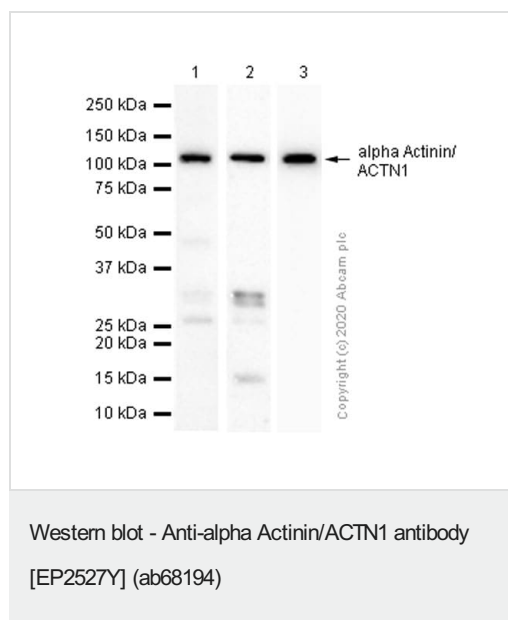


Immunocytochemistry/ Immunofluorescence - Anti-alpha Actinin/ACTN1 antibody [EP2527Y] (ab68194)

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.



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

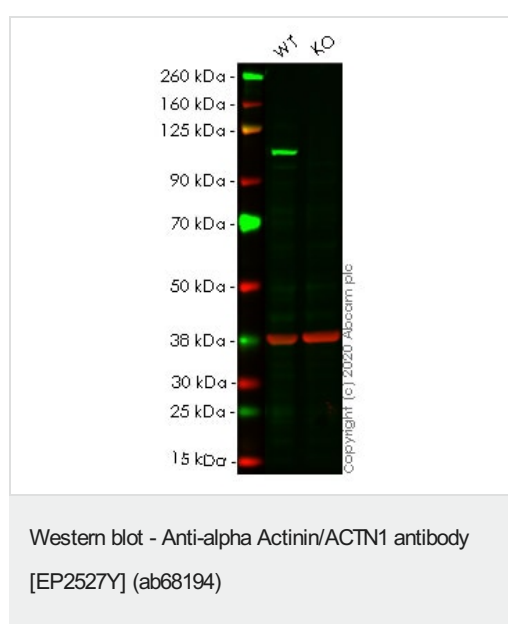
Secondary

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



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.

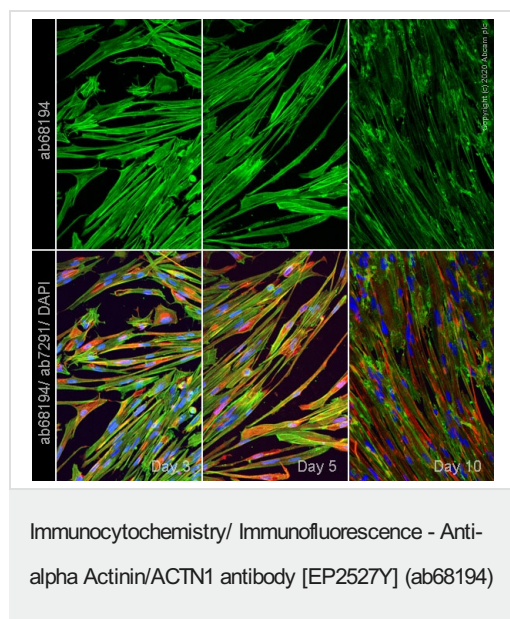
Performed under reducing conditions.

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.

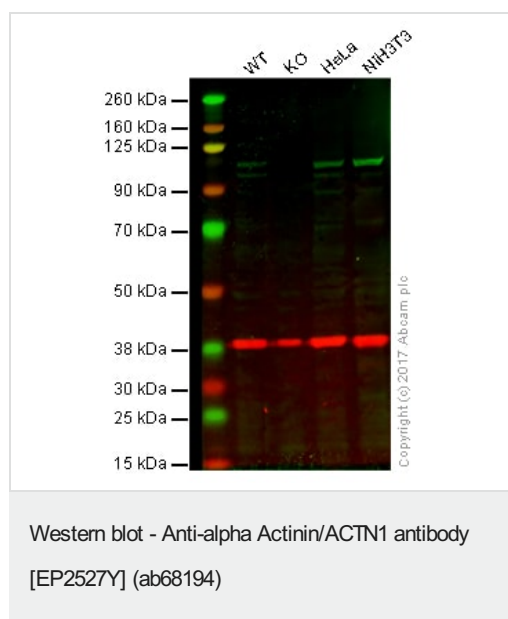
ab68194 was shown to react with alpha Actinin/ACTN1 in wild-type HeLa cells in western blot. Loss of signal was observed when knockout cell line **ab265610** (knockout cell lysate **ab257337**) was used. Wild-type HeLa and ACTN1 knockout HeLa cell lysates were subjected to SDS-PAGE. Membrane was blocked 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.



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.



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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