

# **Product datasheet**

# Anti-alpha Actinin/ACTN1 antibody [EP2528Y] - BSA and Azide free ab185204

KO VALIDATED Recombinant RabMAb

6 Images

Overview		
Product name	Anti-alpha Actinin/ACTN1 antibody [EP2528Y] - BSA and Azide free	
Description	Rabbit monoclonal [EP2528Y] to alpha Actinin/ACTN1 - BSA and Azide free	
Host species	Rabbit	
Tested applications	Suitable for: WB Unsuitable for: Flow Cyt,ICC/IF or IP	
Species reactivity	Reacts with: Mouse, Rat, Human	
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.	
Positive control	WB: HAP1, A431, NIH 3T3, C2C12, C6 and A673 cell lysates and human skeletal muscle and human heart tissue lysates.	
General notes	ab185204 is the carrier-free version of <u>ab81265</u> .	
	Our <u>carrier-free</u> antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency.	
	This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell- based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.	
	Use our <b>conjugation kits</b> for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold.	
	This product is compatible with the Maxpar <sup>®</sup> Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar <sup>®</sup> is a trademark of Fluidigm Canada Inc.	
	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 <u>see here</u> . Our RabMAb <sup>®</sup> technology is a patented hybridoma-based technology for making rabbit	

Pro	perties
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Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C. Do Not Freeze.
Storage buffer	pH: 7.2 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EP2528Y
lsotype	lgG

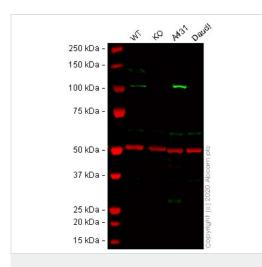
# Applications

The Abpromise guarantee Our <u>Abpromise guarantee</u> covers the use of ab185204 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
WB		Use at an assay dependent concentration. Detects a band of approximately 103 kDa (predicted molecular weight: 103 kDa).
Application notes	Is unsuitable for Flow Cyt, ICC/IF or IP.	
Target		
Function	F-actin cross-linking protein which is thought to anchor actin to a variety of intracellular structures. This is a bundling protein.	
Sequence similarities	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.	
Cellular localization	Cytoplasm > cytoskeleton. Cytoplasm > myofibril > sarcomere > Z line. Colocalizes with MYOZ2 and PPP3CA at the Z-line of heart and skeletal muscle.	

### Images



Western blot - Anti-alpha Actinin/ACTN1 antibody [EP2528Y] - BSA and Azide free (ab185204)

All lanes : Anti-alpha Actinin/ACTN1 antibody [EP2528Y] (ab81265) at 1/1000 dilution

Lane 1 : Wild-type HAP1 cell lysate Lane 2 : ACTN1 knockout HAP1 cell lysate Lane 3 : A431 cell lysate Lane 4 : Daudi cell lysate

Lysates/proteins at 20 µg per lane.

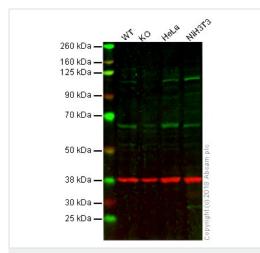
Performed under reducing conditions.

Predicted band size: 103 kDa Observed band size: 115 kDa

This data was developed using the same antibody clone in a different buffer formulation (<u>ab81265</u>).

Lanes 1 - 4: Merged signal (red and green). Green - <u>ab81265</u> observed at 115 kDa. Red - loading control <u>ab7291</u> (Mouse anti-Alpha Tubulin [DM1A]) observed at 55kDa.

**ab81265** was shown to react with ACTN1 in wild-type HAP1 cells in western blot with loss of signal observed in ACTN1 knockout sample. Wild-type and ACTN1 knockout HAP1 cell lysates were subjected to SDS-PAGE. Membranes were blocked in fluorescent western blot (TBS-based) blocking solution before incubation with **ab81265** and **ab7291** (Mouse anti-Alpha Tubulin [DM1A]) overnight at 4°C at a 1 in 1000 Dilution and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Rabbit IgG H&L (IRDye<sup>®</sup> 680RD) preabsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Western blot - Anti-alpha Actinin/ACTN1 antibody [EP2528Y] - BSA and Azide free (ab185204)

This data was developed using <u>ab81265</u>, the same antibody clone in a different buffer formulation.

Lane 1: Wild-type HAP1 whole cell lysate (20 µg)

Lane 2: Alpha Actinin 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 - <u>ab81265</u> observed at 103 kDa. Red - loading control, <u>ab9484</u>, observed at 37 kDa.

**ab81265** was shown to recognize alpha Actinin in wild-type HAP1 cells as signal was lost at the expected MW in alpha Actinin knockout cells. Additional cross-reactive bands were observed in the wild-type and knockout cells. Wild-type and alpha Actinin knockout samples were subjected to SDS-PAGE. **ab81265** and **ab9484** (Mouse anti-GAPDH loading control) were incubated overnight at 4°C at 1/500 dilution and 1/20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye<sup>®</sup> 800CW) preabsorbed **ab216773** and Goat anti-Mouse IgG H&L (IRDye<sup>®</sup> 680RD) preabsorbed **ab216776** secondary antibodies at 1/20000 dilution for 1 hour at room temperature before imaging.

All lanes : Anti-alpha Actinin/ACTN1 antibody [EP2528Y] (ab81265) at 1/1000 dilution (purified)

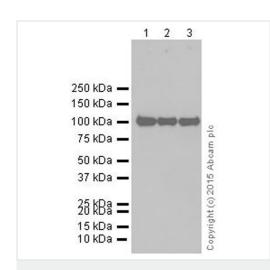
Lane 1 : NIH/3T3 whole cell lysate Lane 2 : C2C12 whole cell lysate Lane 3 : C6 whole cell lysate

Lysates/proteins at 20 µg per lane.

#### Secondary

All lanes : Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/20000 dilution

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



Western blot - Anti-alpha Actinin/ACTN1 antibody [EP2528Y] - BSA and Azide free (ab185204) This data was developed using <u>ab81265</u>, the same antibody clone in a different buffer formulation.

Blocking and dilution buffer: 5% NFDM/TBST.

Anti-alpha Actinin/ACTN1 antibody [EP2528Y] (<u>ab81265</u>) at 1/1000 dilution (purified) + A673 whole cell lysate at 20 µg

## Secondary

Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

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

This data was developed using <u>ab81265</u>, the same antibody clone in a different buffer formulation.

Blocking and dilution buffer: 5% NFDM/TBST.

**All lanes :** Anti-alpha Actinin/ACTN1 antibody [EP2528Y] (<u>ab81265</u>) at 1/1000 dilution (unpurified)

Lane 1 : NIH 3T3 cell lysate Lane 2 : human skeletal muscle tissue lysate Lane 3 : human heart tissue lysate

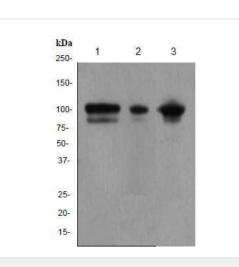
Lysates/proteins at 10 µg per lane.

#### Secondary

All lanes : HRP-conjugated goat anti-rabbit IgG at 1/2000 dilution

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

This data was developed using **<u>ab81265</u>**, the same antibody clone in a different buffer formulation.



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250 kDa 🗕

150 kDa — 100 kDa —

75 kDa —

37 kDa 🗕

28 kBa = 15 kDa = 10 kDa =

Western blot - Anti-alpha Actinin/ACTN1 antibody

[EP2528Y] - BSA and Azide free (ab185204)

Western blot - Anti-alpha Actinin/ACTN1 antibody [EP2528Y] - BSA and Azide free (ab185204)

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Anti-alpha Actinin/ACTN1 antibody [EP2528Y] -

BSA and Azide free (ab185204)

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