abcam

ab217171

Anti-Vinculin antibody [EPR8185] - BSA and Azide free

Recombinant | RabMAb

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Constituents: PBS
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Carrier free	Yes
Clone number	EPR8185
Purification technique	Affinity purification Protein A
Concentration	1.014 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IP

Tested

Species	Human
Dilution info	-

Notes	-

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

WB

Tested

Species	Mouse, Rat, Human
Dilution info	-
Notes	-

IHC-P

Not recommended

Species	Mouse, Rat, Human
Dilution info	-
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	-
Notes	We recommend using this antibody with methanol fixation only. Use of paraformaldehyde fixation may result in non-specific signal. Use at a low concentration to minimize background. We have observed the best results in A431 and HeLa cells when using the antibody in the 0.04 µg/mL to 0.008 µg/mL range.

Species	Mouse
Dilution info	-
Notes	-

Species	Rat
Dilution info	-
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	-
Notes	We recommend using this antibody with methanol fixation only. Use of paraformaldehyde fixation may result in non-specific signal. Use at a low concentration to minimize background. We have observed the best results in A431 and HeLa cells when using the antibody in the 0.04 µg/mL to 0.008 µg/mL range.

Species	Mouse
Dilution info	-
Notes	-

Species	Rat
Dilution info	-
Notes	-

Target data

See full target information Vinculin

Function	Actin filament (F-actin)-binding protein involved in cell-matrix adhesion and cell-cell adhesion. Regulates cell-surface E-cadherin expression and potentiates mechanosensing by the E-cadherin complex. May also play important roles in cell morphology and locomotion.

Storage

Shipped at conditions	Blue Ice
Appropriate short-term storage conditions	+4°C

Appropriate long-term storage conditions	+4°C
Storage information	Do Not Freeze

Notes

ab217171 is the carrier-free version of ab129002.

Patented technology
Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

What are the advantages of a recombinant monoclonal antibody?
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 batch production

For more information, read more on recombinant antibodies.

Conjugation ready
Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. 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 conjugation kits 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.

Compatibility
This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

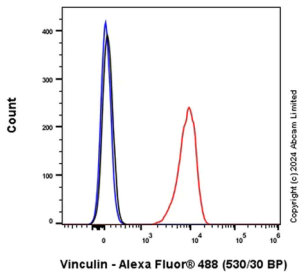
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

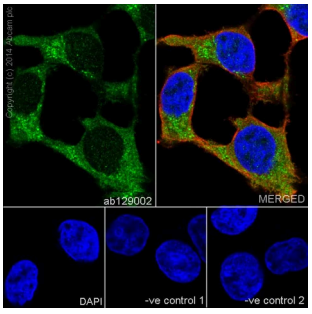
13 product images



Flow Cytometry (Intracellular) - Anti-Vinculin antibody [EPR8185] - BSA and Azide free (ab217171)

Vinculin Flow Cytometry (Intracellular) staining of HEK-293 (human embryonic kidney epithelial cells) using rabbit Anti-Vinculin antibody

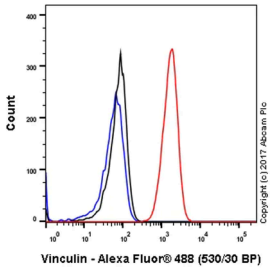
This data was developed using ab196454, the same antibody clone in a different buffer formulation.
Flow cytometry overlay histogram of 4% paraformaldehyde HEK-293 (human embryonic kidney epithelial cell) cells permeabilised with 90% methanol labelling Vinculin with ab196454 at 1/500 dilution (Red line) compared with a Rabbit monoclonal IgG (ab172730) as Isotype control (Black line) and an unlabelled control (cells without incubation with primary antibody) (Blue line).



Immunocytochemistry/ Immunofluorescence - Anti-Vinculin antibody [EPR8185] - BSA and Azide free (ab217171)

Vinculin Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Vinculin antibody

Immunofluorescent staining of HEK293 cells (fixed in 4% PFA, permeabilized with 0.1% Triton X 100) using purified ab129002 at a dilution of 1/50. An Alexa Fluor® 488 goat anti-rabbit antibody (ab150077) was used as the secondary at a dilution of 1/1000 and the cells were counter stained with DAPI. The negative controls are shown in the bottom middle and right hand panels. For negative control 1, the primary was used and then goat anti-mouse IgG was used at a dilution of 1/500. For negative control 2, a mouse primary antibody (ab7291) and anti-rabbit secondary antibody (ab150077) were used.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab129002).

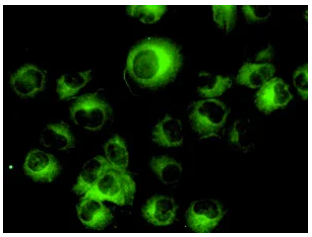


Flow Cytometry (Intracellular) - Anti-Vinculin antibody [EPR8185] - BSA and Azide free (ab217171)

Vinculin Flow Cytometry (Intracellular) staining using rabbit Anti-Vinculin antibody

Intracellular Flow Cytometry analysis of 293 (human embryonic kidney epithelial) cells labeling Vinculin (red) with ab129002 at a 1/200 dilution. Cells were fixed with 4% paraformaldehyde and permeabilized with 90% methanol. A goat anti-rabbit IgG (Alexa Fluor® 488) (ab150077) was used

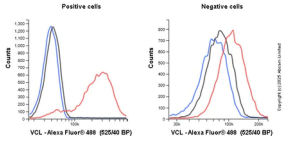
as the secondary antibody at a 1/2000 dilution. Black - Rabbit monoclonal IgG (ab172730). Blue (unlabeled control) - Cells without incubation with the primary and secondary antibodies. This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab129002).



Immunocytochemistry/ Immunofluorescence - Anti-Vinculin antibody [EPR8185] - BSA and Azide free (ab217171)

Vinculin Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Vinculin antibody

Immunofluorescent staining of vinculin in HUVEC cells with unpurified ab129002 at 1/100 dilution. This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab129002).



Flow Cytometry (Intracellular) - Anti-Vinculin antibody [EPR8185] - BSA and Azide free (ab217171)

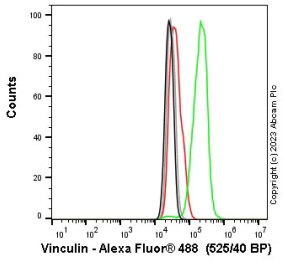
Vinculin Flow Cytometry (Intracellular) staining using rabbit Anti-Vinculin antibody

This data was developed using ab129002, the same antibody clone in a different buffer formulation.
Flow cytometry overlay histogram showing left HeLa positive cells and right negative HeLa VCL KO stained with ab129002 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilised with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS containing 10% normal goat serum to block non-specific protein-protein interaction followed by the antibody (ab129002) (1x 10^6 in 100µl at 0.008µg/ml (1/7875)) for 30min at 22°C.

The secondary antibody Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed was incubated at 1/4000 for 30min at 22°C

Isotype control antibody (black line) was Recombinant Rabbit IgG, monoclonal [EPR25A] - Isotype Control used at the same concentration and conditions as the primary antibody. Unlabelled sample (blue line) was also used as a control.

Acquisition of >5000 events were collected using a 50 mW Blue laser (488nm) and 525/40 bandpass filter.



Flow Cytometry (Intracellular) - Anti-Vinculin antibody [EPR8185] - BSA and Azide free (ab217171)

Vinculin Flow Cytometry (Intracellular) staining using rabbit Anti-Vinculin antibody

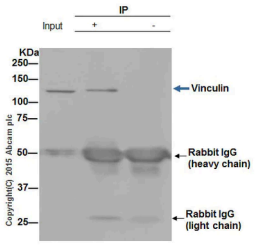
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab129002).

Flow cytometry overlay histogram showing wild-type A-431 (green line) and VCL knockout A-431 stained with ab129002 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilised with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS containing 10% normal goat serum to block non-specific protein-protein interaction followed by the antibody (ab129002) (1x 106 in 100µl at 0.00032 µg/ml (1/6249999)) for 30min at 22°C.

The secondary antibody Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed was incubated at 1/4000 for 30min at 22°C

Isotype control antibody Recombinant Rabbit IgG, monoclonal [EPR25A] - Isotype Control was used at the same concentration and conditions as the primary antibody (wild-type A-431 - black line, VCL knockout A-431 - grey line). Unlabelled sample was also used as a control (this line is not shown for the purpose of simplicity).

Acquisition of >5000 events were collected using a 50 mW Blue laser (488nm) and 525/40 bandpass filter.



Immunoprecipitation - Anti-Vinculin antibody [EPR8185] - BSA and Azide free (ab217171)

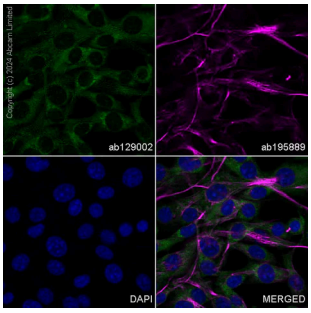
ab129002 (purified) at 1/20 immunoprecipitating vinculin in HeLa cells. Lane 1: HeLa whole cell lysate (10 µg). Lane 2: HeLa whole cell lysate (10 µg). Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab129002 in HeLa whole cell lysate. For western blotting, a HRP-conjugated goat anti-rabbit antibody was used as the secondary antibody (1/1000).
Blocking buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM /TBST.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab129002).

All lanes:
Immunoprecipitation - Anti-Vinculin antibody [EPR8185] - Loading Control (ab129002)

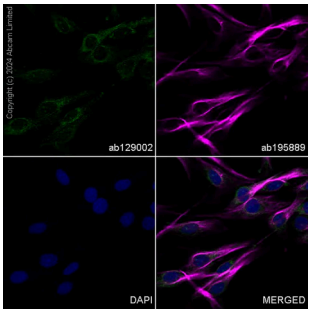
Predicted band size: 124 kDa



Immunocytochemistry/ Immunofluorescence - Anti-Vinculin antibody [EPR8185] - BSA and Azide free (ab217171)

Vinculin Immunocytochemistry/ Immunofluorescence staining of NIH/3T3 (mouse embryonic fibroblasts) using rabbit Anti-Vinculin antibody

This data was developed using ab196454, the same antibody clone in a different buffer formulation.
Immunofluorescent analysis of 100% methanol fixed NIH/3T3 (mouse embryonic fibroblasts) permeabilized using 0.1% Triton X-100 labelling Vinculin with ab196454 at 1/50 dilution (green). ab195889 Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor® 594) at 1/200 dilution (magenta) was used as a counterstain. Nuclear DNA was labelled with DAPI (shown in blue). Image showing cytoplasmic staining in NIH/3T3 cell line was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

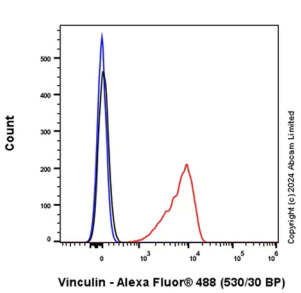


Immunocytochemistry/ Immunofluorescence - Anti-Vinculin antibody [EPR8185] - BSA and Azide free (ab217171)

Vinculin Immunocytochemistry/ Immunofluorescence staining of C6 (rat glial tumor glial cells) using rabbit Anti-Vinculin antibody

This data was developed using ab196454, the same antibody clone in a different buffer formulation.
Immunofluorescent analysis of 100% methanol fixed C6 (rat glial tumor glial cell) permeabilized using 0.1% Triton X-100 labelling Vinculin with ab196454 used at 1/50 dilution (green). ab195889 Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor® 594) at 1/200

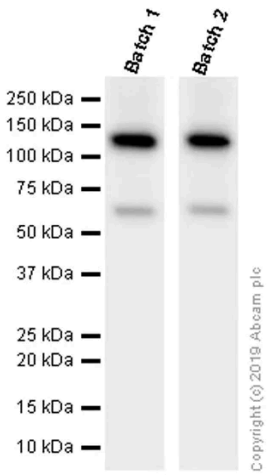
dilution (magenta) was used as a counterstain. Nuclear DNA was labelled with DAPI (shown in blue). Image showing cytoplasmic staining in C6 cell line was taken with a confocal microscope (Leica-Microsystems, TCS SP8).



Flow Cytometry (Intracellular) - Anti-Vinculin antibody [EPR8185] - BSA and Azide free (ab217171)

Vinculin Flow Cytometry (Intracellular) staining of C6 (rat glial tumor glial cells) using rabbit Anti-Vinculin antibody

This data was developed using ab196454, the same antibody clone in a different buffer formulation.
Flow cytometry overlay histogram of 4% paraformaldehyde C6 (rat glial tumor glial cell) cells permeabilised with 90% methanol labelling Vinculin with ab196454 at 1/500 dilution (Red line) compared with a Rabbit monoclonal IgG (ab172730) as Isotype control (Black line) and an unlabelled control (cells without incubation with primary antibody) (Blue line).

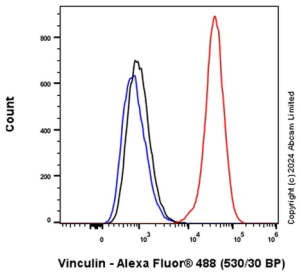


Western blot - Anti-Vinculin antibody [EPR8185] - BSA and Azide free (ab217171)

Vinculin Western blot staining using rabbit Anti-Vinculin antibody

This data was developed using ab129002, the same antibody clone in a different buffer formulation. Different batches of ab129002 were tested on HepG2 (Human hepatocellular carcinoma epithelial cell) lysate at 1.0 µg/ml. 15 µg of lysate was loaded in each lane. Bands observed at 124 kDa.
All lanes:
Western blot - Anti-Vinculin antibody [EPR8185] - Loading Control (ab129002)

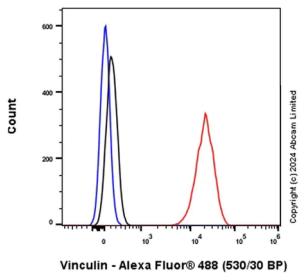
Predicted band size: 124 kDa



Flow Cytometry (Intracellular) - Anti-Vinculin antibody [EPR8185] - BSA and Azide free (ab217171)

Vinculin Flow Cytometry (Intracellular) staining of HUVEC (human umbilical vein endothelial cells) using rabbit Anti-Vinculin antibody

This data was developed using ab196454, the same antibody clone in a different buffer formulation.
Flow cytometry overlay histogram of 4% paraformaldehyde HUVEC (human umbilical vein endothelial cell) cells permeabilised with 90% methanol labelling Vinculin with ab196454 at 1/500 dilution (Red line) compared with a Rabbit monoclonal IgG (ab172730) as Isotype control (Black line) and an unlabelled control (cells without incubation with primary antibody) (Blue line).



Flow Cytometry (Intracellular) - Anti-Vinculin antibody [EPR8185] - BSA and Azide free (ab217171)

Vinculin Flow Cytometry (Intracellular) staining of NIH/3T3 (mouse embryonic fibroblasts) using rabbit Anti-Vinculin antibody

This data was developed using ab196454, the same antibody clone in a different buffer formulation.
Flow cytometry overlay histogram of 4% paraformaldehyde NIH/3T3 (mouse embryonic fibroblast) cells permeabilised with 90% methanol labelling Vinculin with ab196454 at 1/500 dilution (Red line) compared with a Rabbit monoclonal IgG (ab172730) as Isotype control (Black line) and an unlabelled control (cells without incubation with primary antibody) (Blue line).

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.