abcam

Product datasheet

Anti-Vinculin antibody [EPR8185] ab129002

KO VALIDATED Recombinant RabMAb

20 Abreviews 277 References 12 Images

Overview

Product name	Anti-Vinculin antibody [EPR8185]
Description	Rabbit monoclonal [EPR8185] to Vinculin
Host species	Rabbit
Tested applications	**Suitable for:** Flow Cyt (Intra), WB, IP, ICC/IF **Unsuitable for:** IHC-P
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: PC3, HeLa, U937, K562, HUVEC, HepG2 human fetal liver and human fetal kidney lysates ICC/IF: HUVEC cells, HEK-293 cells. IP: HeLa cells Flow Cyt (intra): HEK293 cells, A-431 cells.
General notes	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**.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Stable for 12 months at -20°C.
Storage buffer	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 40% Glycerol, 0.05% BSA, 59% PBS
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR8185

Isotype IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab129002 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
Flow Cyt (Intra)		1/200.
WB	★★★★★ (16)	1/10000 - 1/50000. Detects a band of approximately 124 kDa (predicted molecular weight: 124 kDa).
IP		1/10 - 1/100.
ICC/IF	★★★★★ (2)	1/50 - 1/250.

Application notes Is unsuitable for IHC-P.

Target

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.

Tissue specificity Metavinculin is muscle-specific.

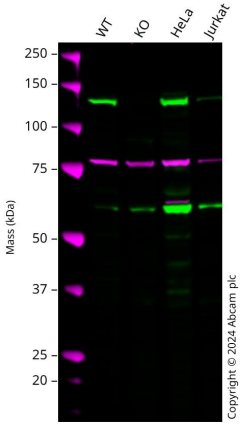
Involvement in disease Defects in VCL are the cause of cardiomyopathy dilated type 1W (CMD1W) [MIM:611407]. Dilated cardiomyopathy is a disorder characterized by ventricular dilation and impaired systolic function, resulting in congestive heart failure and arrhythmia. Patients are at risk of premature death.
Defects in VCL are the cause of cardiomyopathy familial hypertrophic type 15 (CMH15) [MIM:613255]. It is a hereditary heart disorder characterized by ventricular hypertrophy, which is usually asymmetric and often involves the interventricular septum. The symptoms include dyspnea, syncope, collapse, palpitations, and chest pain. They can be readily provoked by exercise. The disorder has inter- and intrafamilial variability ranging from benign to malignant forms with high risk of cardiac failure and sudden cardiac death.

Sequence similarities Belongs to the vinculin/alpha-catenin family.

Domain Exists in at least two conformations. When in the closed, 'inactive' conformation, extensive interactions between the head and tail domains prevent detectable binding to most of its ligands. It takes on an 'active' conformation after cooperative and simultaneous binding of two different ligands. This activation involves displacement of the head-tail interactions and leads to a significant accumulation of ternary complexes. The active form then binds a number of proteins that have both signaling and structural roles that are essential for cell adhesion.
The N-terminal globular head (Vh) comprises of subdomains D1-D4. The C-terminal tail (Vt) binds F-actin and cross-links actin filaments into bundles. An intramolecular interaction between Vh and Vt masks the F-actin-binding domain located in Vt. The binding of talin and alpha-actinin to the D1 subdomain of vinculin induces a helical bundle conversion of this subdomain, leading to the disruption of the intramolecular interaction and the exposure of the cryptic F-actin-binding domain of Vt. Vt inhibits actin filament barbed end elongation without affecting the critical concentration of

	actin assembly.
Post-translational modifications	Phosphorylated; on serines, threonines and tyrosines. Phosphorylation on Tyr-1133 in activated platelets affects head-tail interactions and cell spreading but has no effect on actin binding nor on localization to focal adhesion plaques. Aceylated; mainly by myristic acid but also small amount of palmitic acid.
Cellular localization	Cytoplasm > cytoskeleton. Cell junction > adherens junction. Cell membrane. Cytoplasmic face of adhesion plaques. Recruitment to cell-cell junctions occurs in a myosin II-dependent manner. Interaction with CTNNB1 is necessary for its localization to the cell-cell junctions.

Images



Western blot - Anti-Vinculin antibody [EPR8185] (ab129002)

All lanes : Anti-Vinculin antibody [EPR8185] (ab129002) at 1/10000 dilution

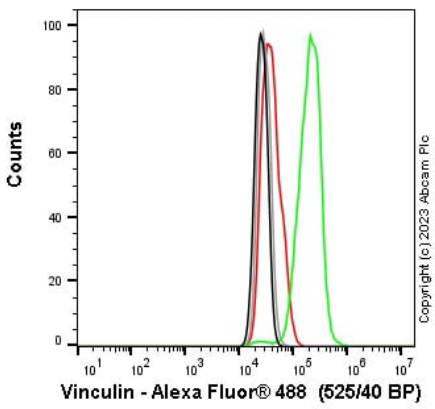
Lane 1 : Wild-type A431 cell lysate
Lane 2 : VCL knockout A431 cell lysate
Lane 3 : HeLa cell lysate
Lane 4 : Jurkat cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 124 kDa
Observed band size: 124 kDa

Western blot: Anti-VCL antibody [EPR8185] (ab129002) staining at 1/10000 dilution, shown in green; Mouse anti-CANX [CANX/1543] (**ab238078**) loading control staining at 1/20000 dilution, shown in magenta. In Western blot, ab129002 was shown to bind specifically to VCL. A band was observed at 124 kDa in wild-type A431 cell lysates with no signal observed at this size in VCL knockout cell line. To generate this image, wild-type and VCL knockout A431 cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution.



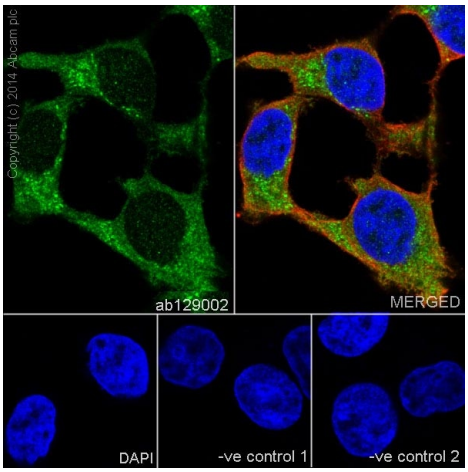
Flow Cytometry (Intracellular) - Anti-Vinculin antibody [EPR8185] (ab129002)

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 10^6 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

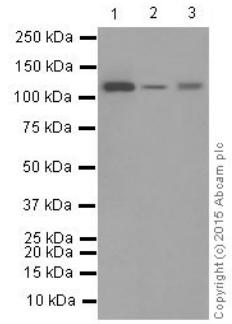
Isotype control antibody Recombinant Rabbit IgG, monoclonal [EPR25A] - Isotype Control was used at the same concentration and conditions as the primary antibody (wild-type A-431 - black line, VCL knockout A-431 - grey line). Unlabelled sample was also used as a control (this line is not shown for the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

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



Immunocytochemistry/ Immunofluorescence - Anti-Vinculin antibody [EPR8185] (ab129002)

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.



Western blot - Anti-Vinculin antibody [EPR8185] (ab129002)

All lanes : Anti-Vinculin antibody [EPR8185] (ab129002) at 1/10000 dilution (purified)

Lane 1 : HepG2 cell lysate
Lane 2 : HeLa cell lysate
Lane 3 : PC3 cell lysate

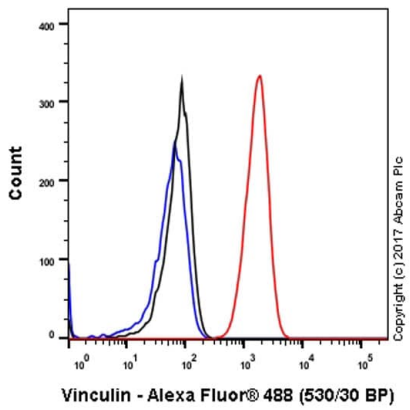
Lysates/proteins at 20 µg per lane.

Secondary
All lanes : HRP goat anti-rabbit (H+L) at 1/1000 dilution

Predicted band size: 124 kDa
Observed band size: 124 kDa

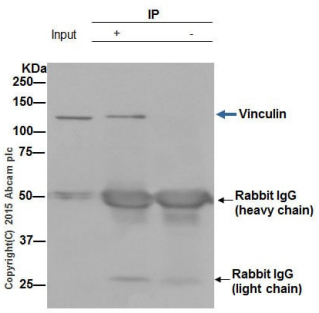
Blocking buffer: 5% NFDM/TBST

Dilution buffer: 5% NFDM/TBST



Flow Cytometry (Intracellular) - Anti-Vinculin antibody [EPR8185] (ab129002)

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 Fluorr® 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.

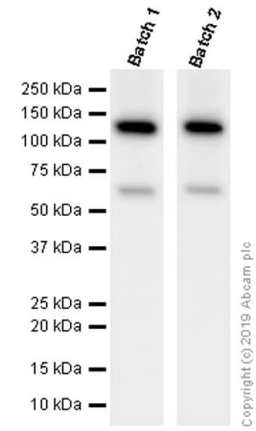


Immunoprecipitation - Anti-Vinculin antibody [EPR8185] (ab129002)

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).

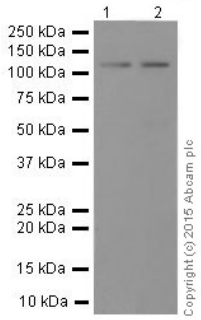
Blocking buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM /TBST.



Western blot - Anti-Vinculin antibody [EPR8185] (ab129002)

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.



Western blot - Anti-Vinculin antibody [EPR8185] (ab129002)

All lanes : Anti-Vinculin antibody [EPR8185] (ab129002) at 1/20000 dilution (purified)

Lane 1 : C6 cell lysate

Lane 2 : rat kidney cell lysate

Lysates/proteins at 10 µg per lane.

Secondary

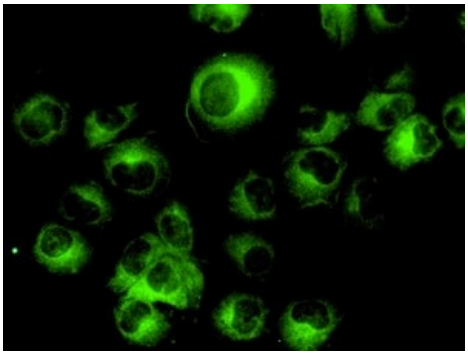
All lanes : HRP goat anti-rabbit (H+L) at 1/1000 dilution

Predicted band size: 124 kDa

Observed band size: 124 kDa

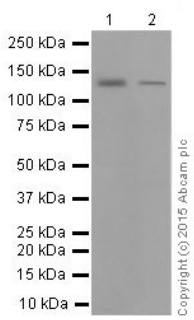
Blocking buffer: 5% NFDM/TBST

Dilution buffer: 5% NFDM/TBST



Immunocytochemistry/ Immunofluorescence - Anti-Vinculin antibody [EPR8185] (ab129002)

Immunofluorescent staining of vinculin in HUVEC cells with unpurified ab129002 at 1/100 dilution.



Western blot - Anti-Vinculin antibody [EPR8185] (ab129002)

All lanes : Anti-Vinculin antibody [EPR8185] (ab129002) at 1/10000 dilution (purified)

Lane 1 : mouse spleen
Lane 2 : NIH/3T3 cell lysate

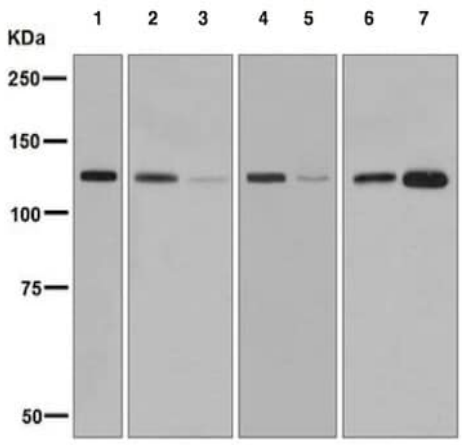
Lysates/proteins at 10 µg per lane.

Secondary
All lanes : HRP goat anti-rabbit (H+L) at 1/1000 dilution

Predicted band size: 124 kDa
Observed band size: 124 kDa

Blocking buffer: 5% NFDM/TBST

Dilution buffer: 5% NFDM/TBST



Western blot - Anti-Vinculin antibody [EPR8185] (ab129002)

All lanes : Anti-Vinculin antibody [EPR8185] (ab129002) at 1/10000 dilution (unpurified)

Lane 1 : PC3 cell lysate
Lane 2 : HeLa cell lysate
Lane 3 : U937 cell lysate
Lane 4 : K562 cell lysate
Lane 5 : HUVEC cell lysate
Lane 6 : Human fetal liver lysate
Lane 7 : Human fetal kidney lysate

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Goat anti-Rabbit HRP at 1/2000 dilution

Predicted band size: 124 kDa
Observed band size: 124 kDa

Anti-Vinculin antibody [EPR8185] (ab129002)

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