abcam

Product datasheet

Anti-Vinculin antibody [EPR20407] ab219649

KO VALIDATED Recombinant RabMAb

7 References 11 Images

Overview

Product name	Anti-Vinculin antibody [EPR20407]
Description	Rabbit monoclonal [EPR20407] to Vinculin
Host species	Rabbit
Tested applications	**Suitable for:** WB, IHC-P
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: PC-3, HeLa, K562, C6, A431, and PC-12 whole cell lysates; Human fetal kidney, fetal heart, testis and fetal liver lysates; Mouse heart and spleen lysates; Rat heart, kidney and spleen lysates. IHC-P: Human breast, colon, gastric cancer and prostate cancer tissues; Mouse colon tissue; Rat stomach tissue.
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 long term. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR20407

Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab219649 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

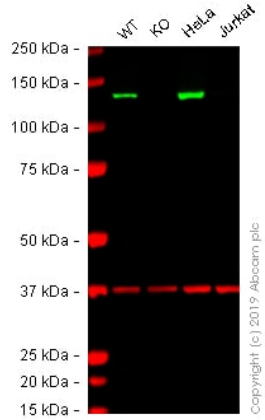
Application	Abreviews	Notes
WB		1/1000. Detects a band of approximately 123 kDa (predicted molecular weight: 124 kDa).
IHC-P		1/2000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

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 [EPR20407] (ab219649)

All lanes : Anti-Vinculin antibody [EPR20407] (ab219649) at 1/1000 dilution

Lane 1 : Wild-type A-431 (Human epidermoid carcinoma cell line) whole cell lysate
Lane 2 : VCL knockout A-431 (Human epidermoid carcinoma cell line) whole cell lysate
Lane 3 : HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate
Lane 4 : Jurkat (Human T cell leukemia cell line from peripheral blood) whole cell lysate

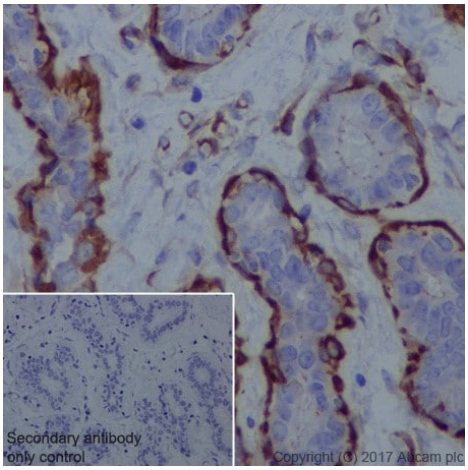
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

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

Lanes 1 - 4: Merged signal (red and green). Green - ab219649 observed at 124 kDa. Red - loading control, **ab8245** (Mouse anti-GAPDH antibody [6C5]) observed at 37kDa.

ab219649 was shown to react with VCL in A431 wild-type cells in Western blot. Loss of signal was observed when VCL knockout sample was used. A431 wild-type and VCL knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3% Milk in TBS-T (0.1% Tween®) before incubation with ab219649 and **ab8245** (Mouse anti-GAPDH antibody [6C5]) overnight at 4°C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Vinculin antibody [EPR20407] (ab219649)

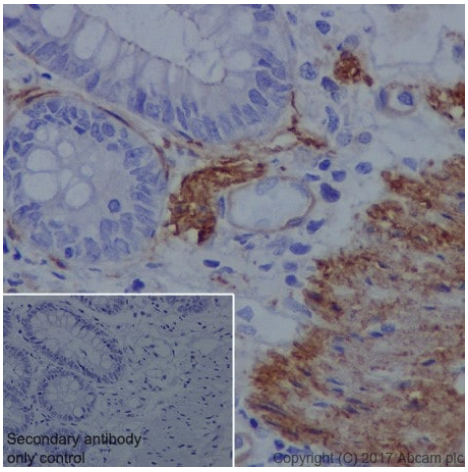
Immunohistochemical analysis of paraffin-embedded human breast tissue labeling Vinculin with ab219649 at 1/2000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use.

Membranous staining on myoepithelium of human breast is observed.

Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) Ready to use.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Vinculin antibody [EPR20407] (ab219649)

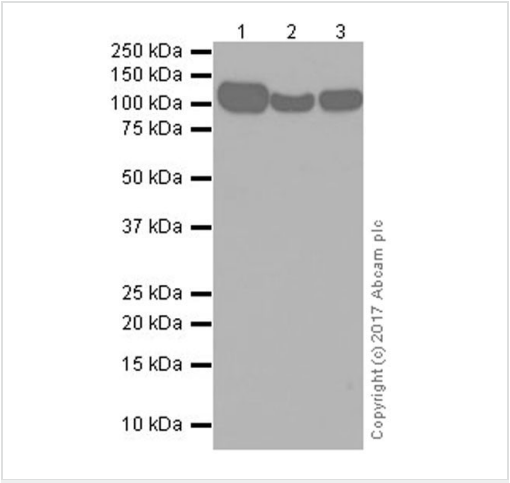
Immunohistochemical analysis of paraffin-embedded human colon tissue labeling Vinculin with ab219649 at 1/2000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use.

Membranous and cytoplasmic staining on smooth muscle cells of human colon is observed.

Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) Ready to use.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Western blot - Anti-Vinculin antibody [EPR20407] (ab219649)

All lanes : Anti-Vinculin antibody [EPR20407] (ab219649) at 1/1000 dilution

Lane 1 : PC-3 (Human prostate adenocarcinoma cell line) whole cell lysate
Lane 2 : HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate
Lane 3 : K562 (Human chronic myelogenous leukemia cell line from bone marrow) whole cell lysate

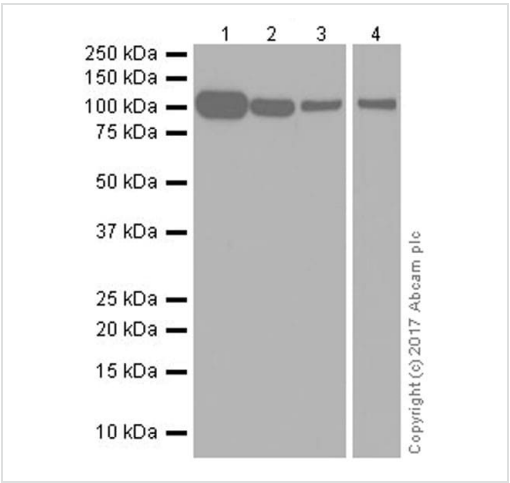
Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

Predicted band size: 124 kDa
Observed band size: 123 kDa

Exposure time: 15 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.



Western blot - Anti-Vinculin antibody [EPR20407] (ab219649)

All lanes : Anti-Vinculin antibody [EPR20407] (ab219649) at 1/1000 dilution

Lane 1 : Human fetal kidney lysate
Lane 2 : Human fetal heart lysate
Lane 3 : Human testis lysate
Lane 4 : Human fetal liver lysate

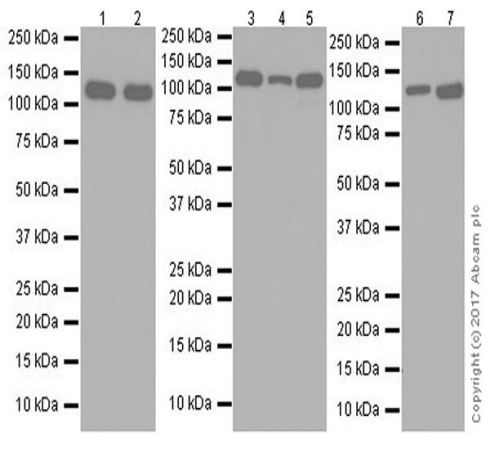
Lysates/proteins at 20 µg per lane.

Secondary
All lanes : VeriBlot for IP Detection Reagent (HRP) (**ab131366**) at 1/4000 dilution

Predicted band size: 124 kDa
Observed band size: 123 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure time: Lane 1-3: 15 seconds; Lane 4: 30 seconds.



Western blot - Anti-Vinculin antibody [EPR20407] (ab219649)

All lanes : Anti-Vinculin antibody [EPR20407] (ab219649) at 1/1000 dilution

Lane 1 : Mouse heart lysate
Lane 2 : Mouse spleen lysate
Lane 3 : Rat heart lysate
Lane 4 : Rat kidney lysate
Lane 5 : Rat spleen lysate
Lane 6 : C6 (Rat glial tumor cell line) whole cell lysate
Lane 7 : PC-12 (Rat adrenal gland pheochromocytoma cell line) whole cell lysate

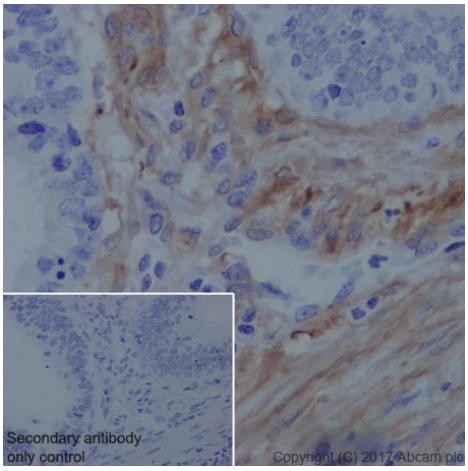
Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

Predicted band size: 124 kDa
Observed band size: 123 kDa

Exposure time: 4 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Vinculin antibody [EPR20407] (ab219649)

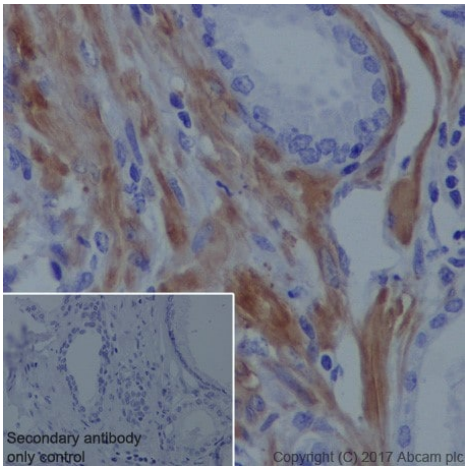
Immunohistochemical analysis of paraffin-embedded human gastric cancer tissue labeling Vinculin with ab219649 at 1/2000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use.

Membranous and cytoplasmic staining on smooth muscle cells of human gastric cancer is observed.

Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) Ready to use.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Vinculin antibody [EPR20407] (ab219649)

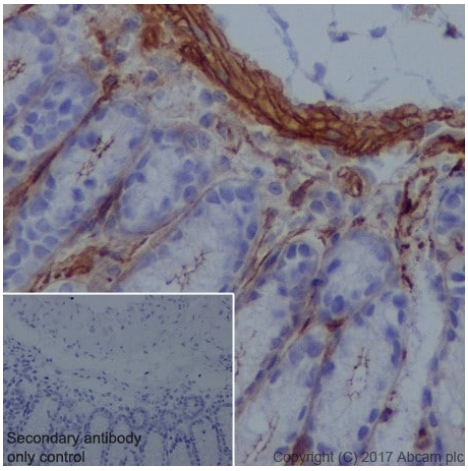
Immunohistochemical analysis of paraffin-embedded human prostate cancer tissue labeling Vinculin with ab219649 at 1/2000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use.

Membranous and cytoplasmic staining on smooth muscle cells of human prostate cancer is observed.

Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) Ready to use.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Vinculin antibody [EPR20407] (ab219649)

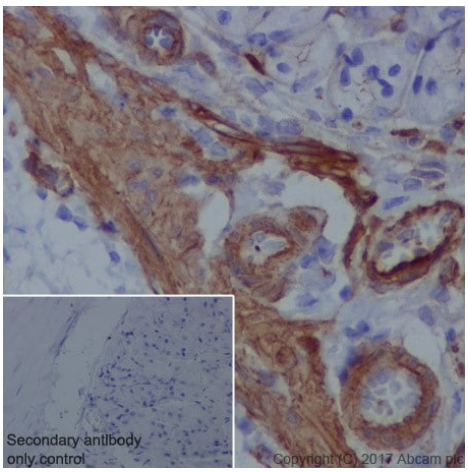
Immunohistochemical analysis of paraffin-embedded mouse colon tissue labeling Vinculin with ab219649 at 1/2000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use.

Membranous and cytoplasmic staining on smooth muscle cells of mouse colon is observed.

Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) Ready to use.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Vinculin antibody [EPR20407] (ab219649)

Immunohistochemical analysis of paraffin-embedded rat stomach tissue labeling Vinculin with ab219649 at 1/2000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use.

Membranous and cytoplasmic staining on smooth muscle cells of rat stomach is observed.

Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) Ready to use.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Anti-Vinculin antibody [EPR20407] (ab219649)

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