abcam

Product datasheet

Anti-Vinculin (phospho Y822) antibody ab200825

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Overview

Product name	Anti-Vinculin (phospho Y822) antibody
Description	Rabbit polyclonal to Vinculin (phospho Y822)
Host species	Rabbit
Tested applications	**Suitable for:** IHC-P, WB
Species reactivity	**Reacts with:** Mouse, Rat, Recombinant fragment **Predicted to work with:** Human
Immunogen	Synthetic peptide corresponding to Human Vinculin (phospho Y822). chemically synthesized phosphopeptide derived from a region of human vinculin that contains tyrosine 822. Database link: **P18206-1**
Positive control	WB: Chick Embryo Fibroblasts (CEFs) transfected with activated Src; IHC-P: Mouse and rat heart tissues.
General notes	The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets your needs before purchasing. If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, along with publications, customer reviews and Q&As

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: 7.30 Preservative: 0.05% Sodium azide Constituents: 50% Glycerol (glycerin, glycerine), 0.1% BSA, 49% PBS
Purity	Immunogen affinity purified
Purification notes	ab200825 has been negatively preadsorbed using a non-phosphopeptide corresponding to the site of phosphorylation to remove antibody that is reactive with non-phosphorylated vinculin. The final product is generated by affinity chromatography using a vinculin-derived peptide that is

phosphorylated at tyrosine 822.

Clonality Polyclonal

Isotype IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab200825 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
IHC-P		1/10 - 1/100. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
WB	★★★☆☆ (1)	1/1000. Predicted molecular weight: 124 kDa.

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.

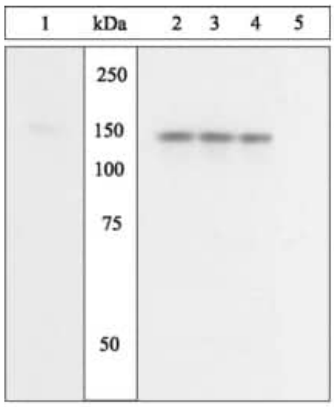
Sequence similarities Belongs to the vinculin/alpha-catenin family.

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 Phosphorylated; on serines, threonines and tyrosines. Phosphorylation on Tyr-1133 in activated

modifications	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 (phospho Y822) antibody (ab200825)

All lanes : Anti-Vinculin (phospho Y822) antibody (ab200825) at 1/1000 dilution

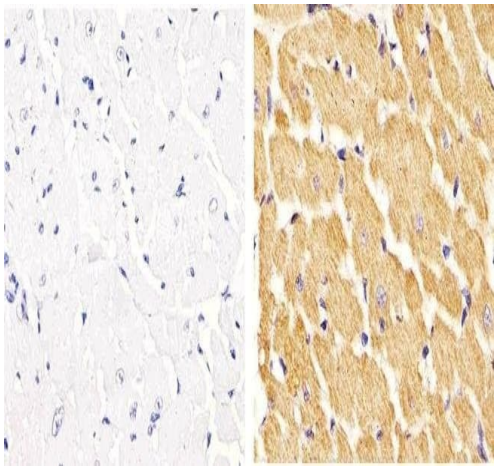
Lane 1 : Untransfected CEF lysates
Lane 2 : Src transfected CEF lysates
Lane 3 : Src transfected CEF lysates with non-phosphopeptide corresponding to immunogen
Lane 4 : Src transfected CEF lysates with generic phosphotyrosine containing peptide
Lane 5 : Src transfected CEF lysates with phosphopeptide immunogen

Secondary
Lanes 1-2 : goat F(ab')2 anti-rabbit IgG HRP conjugate

Developed using the ECL technique.

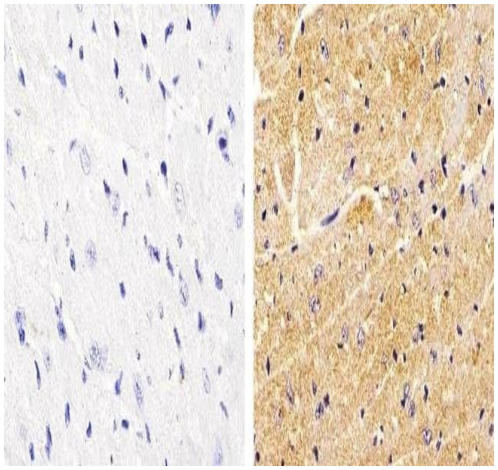
Predicted band size: 124 kDa

10% polyacrylamide gel transferred to PVDF blocked with a 5% BSA-TBST



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Vinculin (phospho Y822) antibody (ab200825)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of rat heart tissue labeling Vinculin (phospho Y822) with ab200825 (right). Heat mediated antigen retrieval was performed using 10mM sodium citrate (pH 6.0), microwaved for 8-15 min. Following antigen retrieval, tissues were blocked in 3% H2O2-methanol for 15 min at room temperature, washed with ddH2O and PBS, and then probed with ab200825 diluted in 3% BSA-PBS at a dilution of 1/20 overnight at 4°C in a humidified chamber. Tissues were washed extensively in PBST and detection was performed using an HRP-conjugated secondary antibody followed by colorimetric detection using a DAB kit. Tissues were counterstained with hematoxylin and dehydrated with ethanol and xylene to prep for mounting. Negative control without primary antibody (left).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Vinculin (phospho Y822) antibody (ab200825)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of mouse heart tissue labeling Vinculin (phospho Y822) with ab200825 (right). Heat mediated antigen retrieval was performed using 10mM sodium citrate (pH 6.0), microwaved for 8-15 min. Following antigen retrieval, tissues were blocked in 3% H2O2-methanol for 15 min at room temperature, washed with ddH2O and PBS, and then probed with ab200825 diluted in 3% BSA-PBS at a dilution of 1/20 overnight at 4°C in a humidified chamber. Tissues were washed extensively in PBST and detection was performed using an HRP-conjugated secondary antibody followed by colorimetric detection using a DAB kit. Tissues were counterstained with hematoxylin and dehydrated with ethanol and xylene to prep for mounting. Negative control without primary antibody (left).

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