abcam

Product datasheet

Anti-Paxillin antibody [E228] ab32115

KO VALIDATED Recombinant RabMAb

8 References 15 Images

Overview

Product name	Anti-Paxillin antibody [E228]
Description	Rabbit monoclonal [E228] to Paxillin
Host species	Rabbit
Specificity	The mouse and rat recommendation is based on the WB results. We do not guarantee IHC-P for mouse and rat.
Tested applications	**Suitable for:** WB, ICC/IF, IHC-P, IP, Flow Cyt (Intra), Dot blot, ELISA
Species reactivity	**Reacts with:** Mouse, Rat, Human, Recombinant fragment
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: HeLa, NIH 3T3, HEK-293, C2C12, A431, PC-3 and Rat-1 whole cell lysates. ICC/IF: HeLa cells. IHC-P: Human colon and breast carcinoma. Flow Cyt (intra): HeLa cells. IP: HEK-293 cell lysate.
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. **We are constantly working hard to ensure we provide our customers with best in class antibodies. As a result of this work we are pleased to now offer this antibody in purified format. We are in the process of updating our datasheets. The purified format is designated 'PUR' on our product labels. If you have any questions regarding this update, please contact our Scientific Support team.**

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles.
Storage buffer	pH: 7.20

	Preservative: 0.01% Sodium azide Constituents: 40% Glycerol (glycerin, glycerine), PBS, 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	E228
Isotype	IgG

Applications

The Abpromise guarantee Our Abpromise guarantee covers the use of ab32115 in the following tested applications.

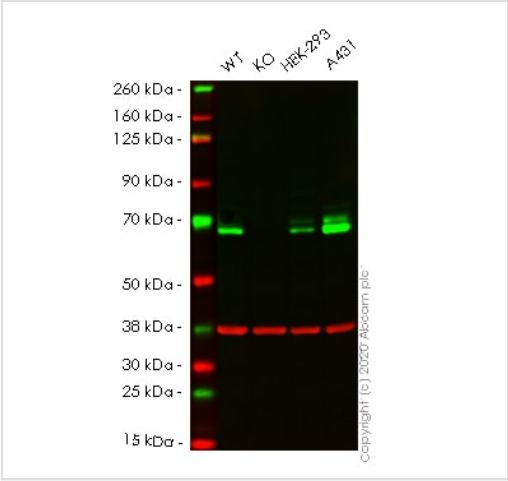
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB		1/10000. Predicted molecular weight: 64 kDa.
ICC/IF		1/100. For unpurified use at 1/250
IHC-P		1/50. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. The mouse and rat recommendation is based on the WB results. We do not guarantee IHC-P for mouse and rat. See IHC antigen retrieval protocols. For unpurified use at 1/100 - 1/250
IP		1/20. For unpurified use at 1/100
Flow Cyt (Intra)		1/20.
Dot blot		1/1000.
ELISA		Use at an assay dependent concentration.

Target

Function	Cytoskeletal protein involved in actin-membrane attachment at sites of cell adhesion to the extracellular matrix (focal adhesion).
Sequence similarities	Belongs to the paxillin family. Contains 4 LIM zinc-binding domains.
Post-translational modifications	Phosphorylated on tyrosine residues during integrin-mediated cell adhesion, embryonic development, fibroblast transformation and following stimulation of cells by mitogens.
Cellular localization	Cytoplasm > cytoskeleton. Cell junction > focal adhesion.

Images



Western blot - Anti-Paxillin antibody [E228] (ab32115)

All lanes : Anti-Paxillin antibody [E228] (ab32115) at 1/10000 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : PXN knockout HeLa cell lysate
Lane 3 : HEK-293 cell lysate
Lane 4 : A431 cell lysate

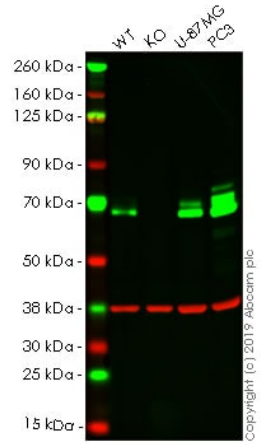
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 64 kDa
Observed band size: 65 kDa

Lanes 1-4: Merged signal (red and green). Green - ab32115 observed at 65 kDa. Red - loading control ab8245 observed at 37 kDa.

ab32115 Anti-Paxillin antibody [E228] was shown to specifically react with Paxillin in wild-type HeLa cells. Loss of signal was observed when knockout cell line ab264767 (knockout cell lysate ab257044) was used. Wild-type and Paxillin knockout samples were subjected to SDS-PAGE. ab32115 and Anti-alpha Tubulin antibody [EP1332Y] - Microtubule Marker (ab52866) were incubated overnight at 4°C at 1 in 10000 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (ab216776) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Western blot - Anti-Paxillin antibody [E228] (ab32115)

All lanes : Anti-Paxillin antibody [E228] (ab32115) at 1/10000 dilution

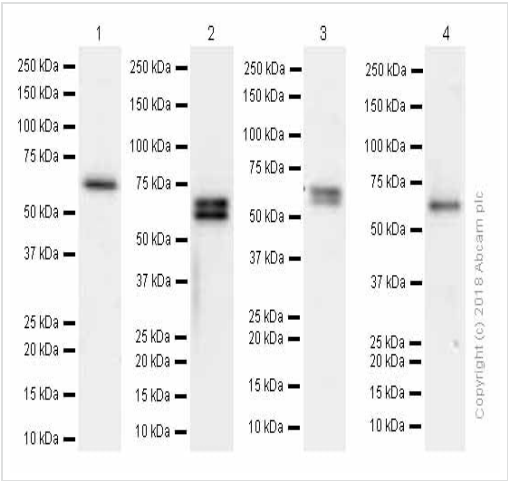
Lane 1 : Wild-type A431 whole cell lysate
Lane 2 : PXN knockout A431 whole cell lysate
Lane 3 : U-87 MG whole cell lysate
Lane 4 : PC-3 whole cell lysate

Lysates/proteins at 20 µg per lane.

Predicted band size: 64 kDa
Observed band size: 65 kDa

Lanes 1 - 4: Merged signal (red and green). Green - ab32115 observed at 65 kDa. Red - loading control, ab8245, observed at 37 kDa.

ab32115 was shown to specifically react with PXN in wild-type A431 cells as signal was lost in PXN knockout cells. Wild-type and PXN knockout samples were subjected to SDS-PAGE. The membrane was blocked with 3% Milk. Ab32115 and ab8245 (Mouse anti GAPDH loading control) were incubated overnight at 4°C at 1/10000 dilution and 1/20000 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 1/20000 dilution for 1 hour at room temperature before imaging.



Western blot - Anti-Paxillin antibody [E228] (ab32115)

All lanes : Anti-Paxillin antibody [E228] (ab32115) at 1/1000 dilution (purified)

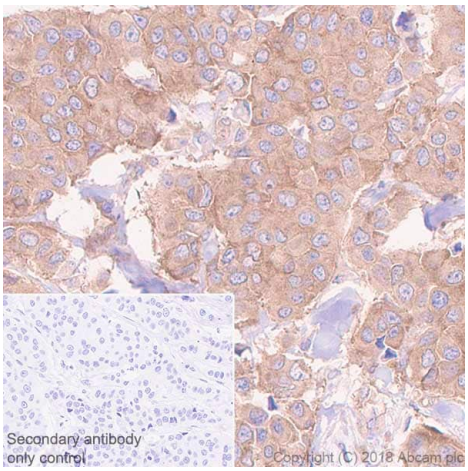
Lane 1 : HEK-293 (Human embryonic kidney) whole cell lysates
Lane 2 : NIH/3T3 (Mouse embryonic fibroblast) whole cell lysates
Lane 3 : C2C12 (Mouse myoblasts myoblast) whole cell lysates
Lane 4 : Rat-1 (Rat embryonic fibroblast) whole cell lysates

Lysates/proteins at 15 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (ab97051)

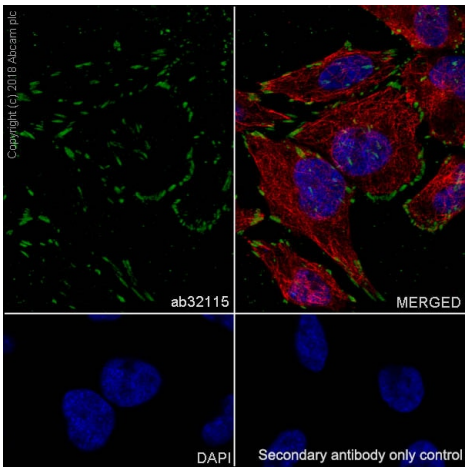
Predicted band size: 64 kDa
Observed band size: 60,64 kDa

Based on the immunogen sequence blast, this antibody recognizes alpha, beta and gamma isoforms. The molecular weight observed is consistent with what has been described in the literature PMID: 9712867 and 20388733



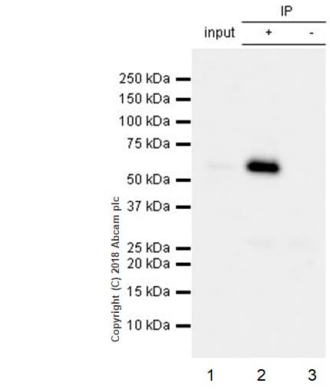
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Paxillin antibody [E228] (ab32115)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Human breast carcinoma tissue sections labeling Paxillin with Purified ab32115 at 1:50 dilution (2.34 µg/ml). Heat mediated antigen retrieval was performed using ab93684 (Tris/EDTA buffer, pH 9.0). ImmunoHistoProbe one step HRP Polymer (ready to use)was used. Negative control:PBS instead of the primary antibody. Hematoxylin was used as a counterstain.



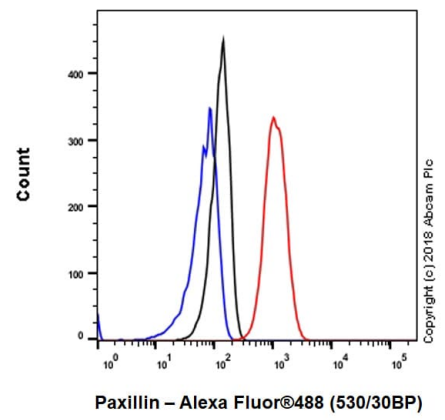
Immunocytochemistry/ Immunofluorescence analysis of HeLa (Human cervix adenocarcinoma epithelial cell) cells labeling Paxillin with Purified ab32115 at 1:100 dilution (1.2 µg/ml). Cells were fixed in 4% Paraformaldehyde and permeabilized with 0.1% tritonX-100. Cells were counterstained with Ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) 1:200 (2.5 µg/ml). Goat anti rabbit IgG (Alexa Fluor® 488, ab150077) was used as the secondary antibody at 1:1000 (2 µg/ml) dilution. DAPI nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.

Immunocytochemistry/ Immunofluorescence - Anti-Paxillin antibody [E228] (ab32115)



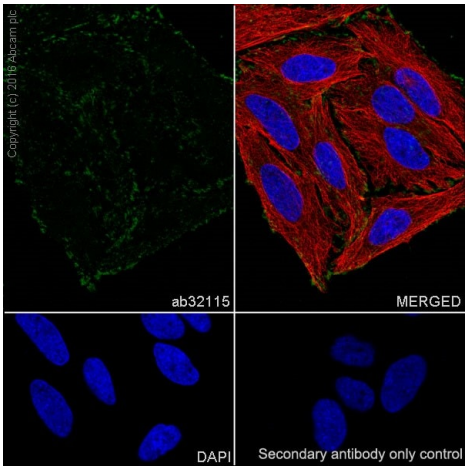
ab32115 (purified) at 1:20 dilution (0.5µg) immunoprecipitating Paxillin in HEK-293 whole cell lysate.
Lane 1 (input): HEK-293 (Human embryonic kidney epithelial cell) whole cell lysate 10µg
Lane 2 (+): ab32115 & HEK-293 whole cell lysate
Lane 3 (-): Rabbit monoclonal IgG (ab172730) instead of ab32115 in HEK-293 whole cell lysate
For western blotting, VeriBlot for IP Detection Reagent (HRP) (ab131366) was used for detection at 1:1000 dilution.
Blocking and diluting buffer: 5% NFDM/TBST.

Immunoprecipitation - Anti-Paxillin antibody [E228] (ab32115)



Intracellular Flow Cytometry analysis of HeLa (Human cervix adenocarcinoma epithelial cell) cells labeling Paxillin with Purified ab32115 at 1/20 dilution (10µg/ml) (red). Cells were fixed with 4% Paraformaldehyde. A Goat anti rabbit IgG (Alexa Fluorr® 488, ab150077) secondary antibody was used at 1/2000. Isotype control - Rabbit monoclonal IgG (Black). Unlabeled control - Cell without incubation with primary antibody and secondary antibody (Blue).

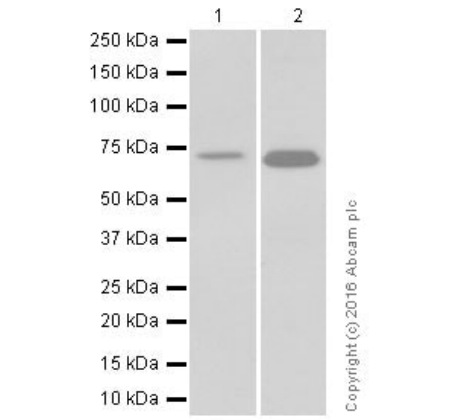
Flow Cytometry (Intracellular) - Anti-Paxillin antibody [E228] (ab32115)



Immunocytochemistry/ Immunofluorescence - Anti-Paxillin antibody [E228] (ab32115)

Immunocytochemistry/ Immunofluorescence analysis of HeLa (Human epithelial cell line from cervix adenocarcinoma) cells labeling Paxillin with ab32115 at 1/100 (1 µg/ml). Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. ab150077, an Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/1000, 2 µg/ml) was used as the secondary antibody. The cells were counterstained with ab195889, anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) at 1/200, 2.5 µg/ml. Nuclei counterstained with DAPI (blue).

Confocal image showing membranous staining on HeLa cells.



Western blot - Anti-Paxillin antibody [E228] (ab32115)

All lanes : Anti-Paxillin antibody [E228] (ab32115) at 1/1000 dilution

Lane 1 : HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysates
Lane 2 : HEK-293 (human embryonic kidney) whole cell lysates

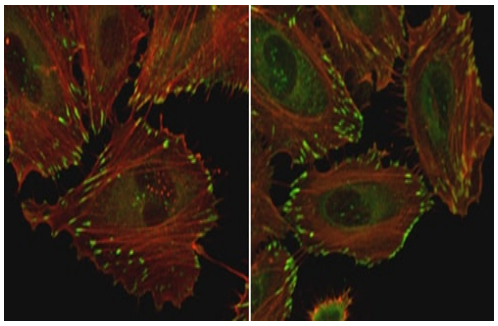
Lysates/proteins at 15 µg per lane.

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

Predicted band size: 64 kDa
Observed band size: 64 kDa

Exposure time: 3 minutes

Blocking and diluting buffer and concentration: 5% NFDM/TBST.

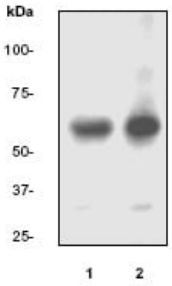


Immunocytochemistry/ Immunofluorescence - Anti-Paxillin antibody [E228] (ab32115)

Image from Beheshti Zavareh R et al., PLoS One. 2012;7(9):e43721. doi: 10.1371/journal.pone.0043721. Epub 2012 Sep 5. Fig 3.; doi:10.1371/journal.pone.0043721; September 5, 2012, PLoS ONE 7(9): e43721.

Immunofluorescence analysis of HeLa cells, staining Paxillin with ab32115.

Cells on the right were treated with MGAT1 shRNA. Cells were fixed with 2% paraformaldehyde, permeabilized using 0.2% Triton-X-100 and blocked by 5% BSA for 1 hour. Cells were incubated with primary antibody (1/400) overnight at 4°C. A FITC-conjugated donkey anti-rabbit IgG (1/500) was used as the secondary antibody.

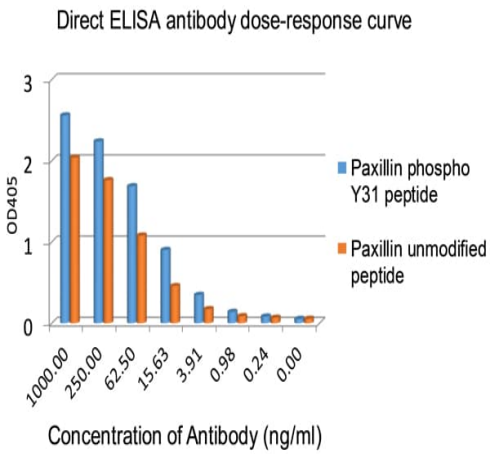


Western blot - Anti-Paxillin antibody [E228] (ab32115)

All lanes : Anti-Paxillin antibody [E228] (ab32115) at 1/10000 dilution

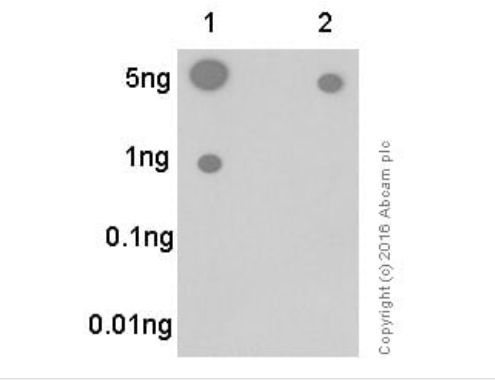
Lane 1 : untreated NIH 3T3 cell lysate
Lane 2 : PDGF treated NIH 3T3 cell lysate

Predicted band size: 64 kDa
Observed band size: 64 kDa



ELISA - Anti-Paxillin antibody [E228] (ab32115)

Direct ELISA antigen dose-response curve using ab32115 at 0-1000 ng/mL. Antigen (human Paxillin phospho Y31 peptide/ unmodified peptide) concentration of 1000 ng/mL. An alkaline phosphatase-conjugated goat anti-rabbit IgG (H+L) (1/2500) was used as the secondary antibody.

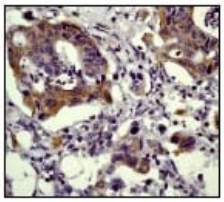


Dot Blot - Anti-Paxillin antibody [E228] (ab32115)

Dot blot analysis of Paxillin (pY31) peptide (Lane 1) and Paxillin non-phospho peptide (Lane 2) labelling Paxillin with ab32115 at a dilution of 1/1000. ab97051 (Peroxidase conjugated goat anti-rabbit IgG (H+L)) was used as the secondary antibody at a dilution of 1/100000.

Blocking and dilution buffer: 5% NFDM/TBST.

Exposure time: 3 minutes.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Paxillin antibody [E228] (ab32115)

Immunohistochemical analysis of Paxillin expression in paraffin-embedded human colon carcinoma using 1/100 ab32115.

Anti-Paxillin antibody [E228] (ab32115)

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