abcam

Product datasheet

Anti-Paxillin antibody [E228] ab32115

KO VALIDATED Recombinant RabMAb

12 References 14 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
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**.

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.

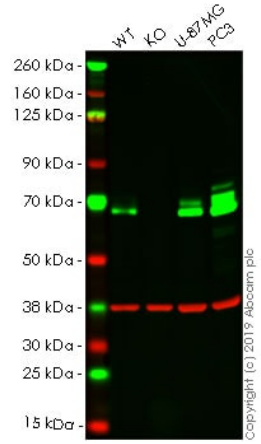
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

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 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.

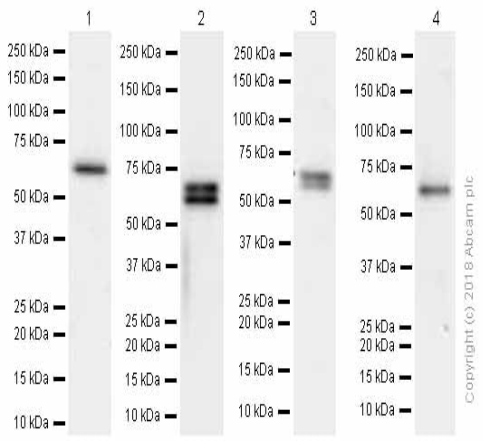
Performed under reducing conditions.

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

Exposure time: 10 seconds

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)

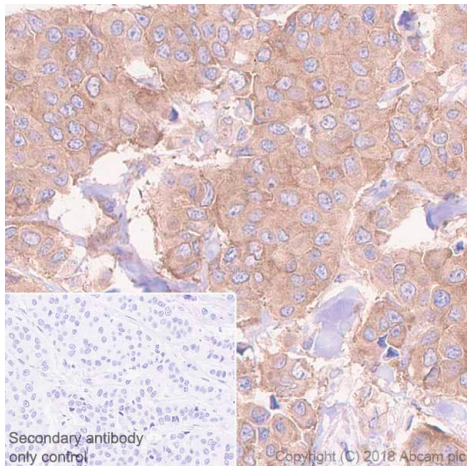
Lane 1 : HEK-293 (Human embryonic kidney) whole cell lysates
Lane 2 : NIH/3T3 (Mouse embryonic fibroblast) whole cell lysates
Lane 3 : C2C12 (Mouse myoblasts myoblast) whole cell lysates
Lane 4 : Rat-1 (Rat embryonic fibroblast) whole cell lysates

Lysates/proteins at 15 µg per lane.

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

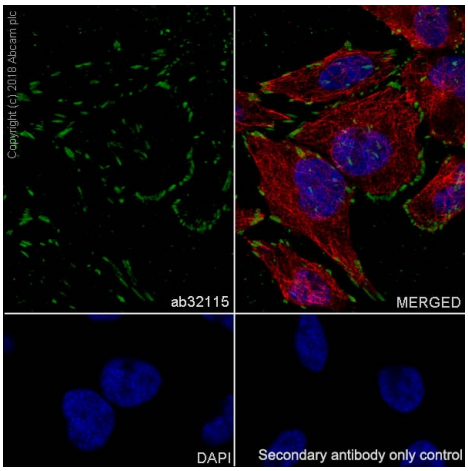
Predicted band size: 64 kDa
Observed band size: 60,64 kDa

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



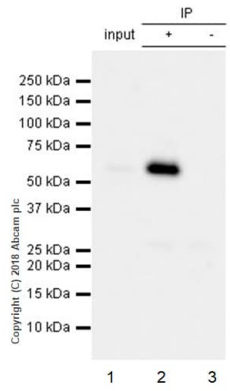
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Paxillin antibody [E228] (ab32115)

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.



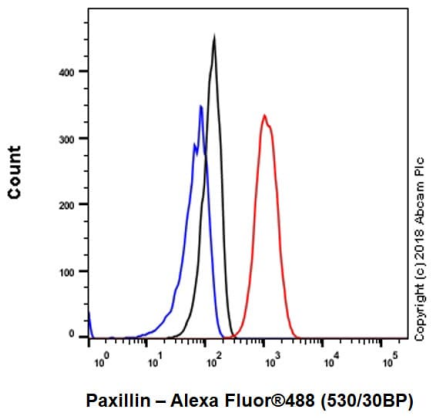
Immunocytochemistry/ Immunofluorescence - Anti-Paxillin antibody [E228] (ab32115)

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.



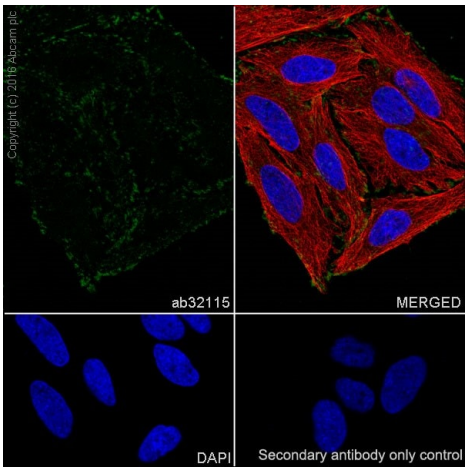
Immunoprecipitation - Anti-Paxillin antibody [E228] (ab32115)

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.



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

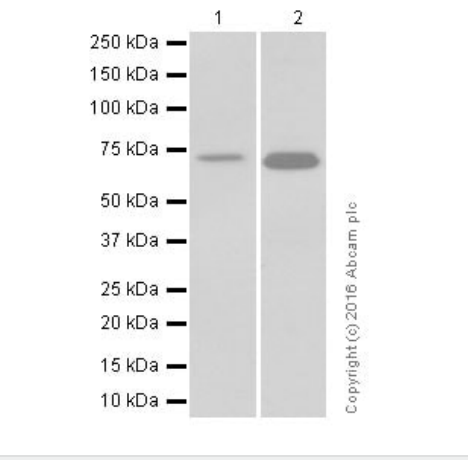
Intracellular Flow Cytometry analysis of HeLa (Human cervix adenocarcinoma epithelial cell) cells labeling Paxillin with Purified ab32115 at 1/20 dilution (10µg/ml) (red). Cells were fixed with 4% Paraformaldehyde. A Goat anti rabbit IgG (Alexa Fluorr® 488, **ab150077**) secondary antibody was used at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



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

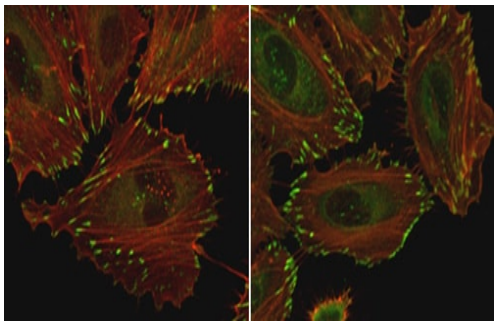
Lysates/proteins at 15 µg per lane.

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.

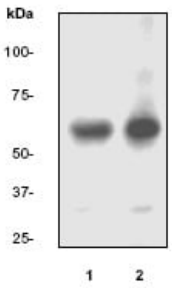


Immunocytochemistry/ Immunofluorescence - Anti-Paxillin antibody [E228] (ab32115)

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.

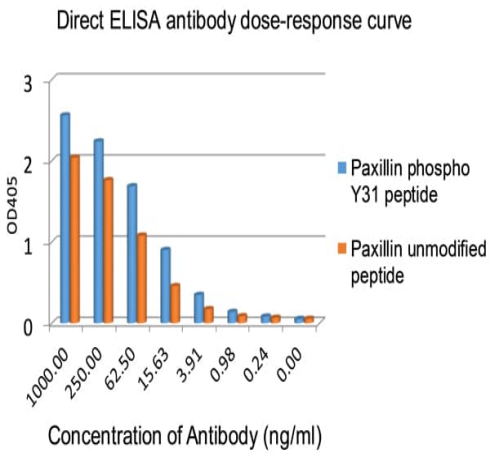


Western blot - Anti-Paxillin antibody [E228] (ab32115)

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

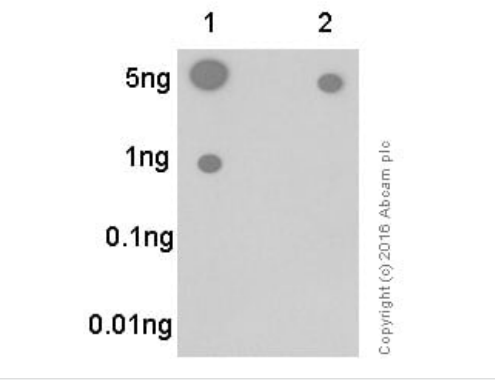
Lane 1 : untreated NIH 3T3 cell lysate
Lane 2 : PDGF treated NIH 3T3 cell lysate

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.

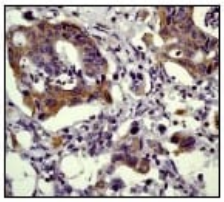


Dot Blot - Anti-Paxillin antibody [E228] (ab32115)

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.

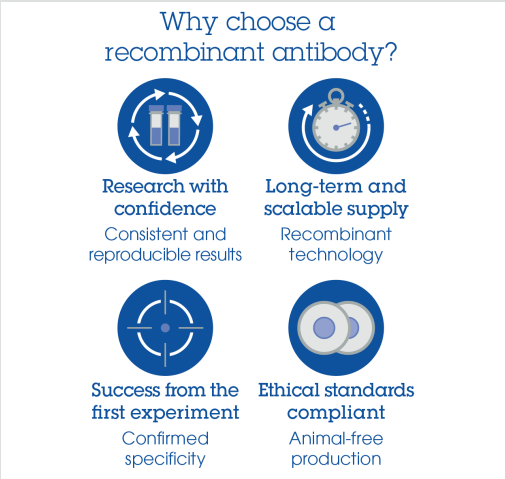
Blocking and dilution buffer: 5% NFDM/TBST.

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)

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit **https://www.abcam.com/abpromise** or contact our technical team.

Terms and conditions

- Guarantee only valid for products bought direct from Abcam or one of our authorized distributors