abcam

ab32115

Anti-Paxillin antibody [E228]

Recombinant | RabMAb | KO Validated | 20ul selling size

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Clone number	E228
Purification technique	Affinity purification Protein A
Concentration	0.12 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IP

Tested

Species	Human
Dilution info	1/20
Notes	For unpurified use at 1/100

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Not recommended

Species	Synthetic peptide - Human
Dilution info	-
Notes	-

ELISA

Tested

Species	Synthetic peptide - Human
Dilution info	-
Notes	-

Expected

Species	Human, Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Dot

Tested

Species	Synthetic peptide - Human
Dilution info	1/1000
Notes	-

Expected

Species	Human, Mouse, Rat

Dilution info	Use at an assay dependent concentration.
Notes	-

WB

Tested

Species	Mouse
Dilution info	1/10000
Notes	-

Species	Rat
Dilution info	1/10000
Notes	-

Species	Human
Dilution info	1/10000
Notes	-

Not recommended

Species	Synthetic peptide - Human
Dilution info	-
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	1/100
Notes	For unpurified use at 1/250

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.

Notes	-

Not recommended

Species	Synthetic peptide - Human
Dilution info	-
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	1/20
Notes	-

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Not recommended

Species	Synthetic peptide - Human
Dilution info	-
Notes	-

IHC-P

Tested

Species	Human
Dilution info	1/50
Notes	For unpurified use at 1/100 - 1/250

Not recommended

Species	Mouse, Rat, Synthetic peptide - Human
Dilution info	-
Notes	-

Target data

See full target information Paxillin

Function	Cytoskeletal protein involved in actin-membrane attachment at sites of cell adhesion to the extracellular matrix (focal adhesion). Recruits other proteins such as TRIM15 to focal adhesion.

Storage

Shipped at conditions	Conditional Ambient
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C
Aliquoting information	Upon delivery aliquot
Storage information	Avoid freeze / thaw cycle

Notes

Patented technology
Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

What are the advantages of a recombinant monoclonal antibody?
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 batch production

For more information, read more on recombinant antibodies.

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	Paxillin also known as PXN is a cytoskeletal protein involved in the assembly of focal adhesions. It has a molecular weight of approximately 68 kDa. You will find Paxillin expressed widely in various tissues including the liver colon and lung. Paxillin plays a part in cell motility by interacting with other focal adhesion proteins. It plays a significant mechanical role by serving as a docking site for structural and signaling molecules.
Biological function summary	Paxillin serves key functions in cellular processes such as migration proliferation and survival. It interacts with different proteins including vinculin and talin forming part of a complex at the focal adhesion sites. The phosphorylation state of Paxillin can modulate its interactions and in this way influence the assembly of signaling complexes that control dynamic cellular processes.
Pathways	Paxillin participates in the integrin signaling and MAPK pathways. It operates by linking integrin receptors to the actin cytoskeleton facilitating signal transduction. Paxillin phosphorylation is an important regulatory mechanism within these pathways. In particular its interaction with focal adhesion kinase (FAK) and Src family kinases signifies its role in transmitting signals from the extracellular matrix to the cellular interior which impacts cell behavior and response.
Associated diseases and disorders	Paxillin has links with osteosarcoma and lung cancer. Deregulation of Paxillin expression or its phosphorylation state contributes to oncogenic transformation and tumor progression. In cancer Paxillin associates with altered activity of proteins such as Src and FAK exacerbating disease states. This relationship highlights Paxillin's potential as a biomarker and therapeutic target in treating related malignancies.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

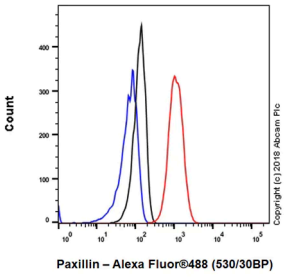
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

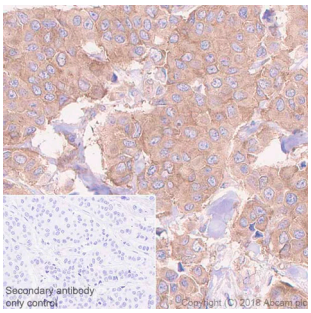
15 product images



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

Paxillin Flow Cytometry (Intracellular) staining using rabbit Anti-Paxillin antibody

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).



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

Paxillin Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-Paxillin antibody

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.

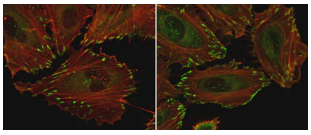
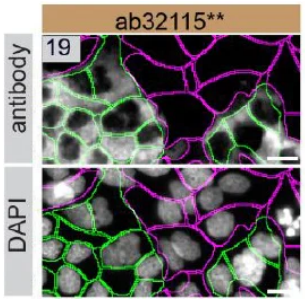


Image from Beheshti Zavareh R et al. PLoS One. 2012;7(9):e43721. doi: 10.1371/journal.pone.0043721. Epub 2012 Sep 5. Fig 3.; doi:10.1371/journal.pone.0043721; September 5 2012 PLoS ONE 7(9): e43721.

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

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.

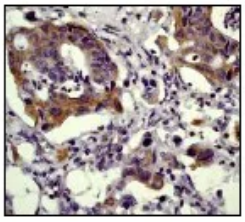


Immunocytochemistry/ Immunofluorescence - Anti-Paxillin antibody [E228] (ab32115)

Paxillin Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Paxillin antibody

ab32115 was shown to react with PXN in wild-type A-431 cells in immunocytochemistry with loss of signal observed in PXN knockout cell line ab261892. Wild-type and knockout cells were mixed and pelleted at a 1:1 ratio on coverslips. The cells were fixed with 4% paraformaldehyde (15 min) then permeabilized with 0.1% Triton X-100 (10min) and then blocked with 1x PBS, 0.01% Triton X-100, 5% BSA, 5% NGS. The cells were then incubated with ab32115 at 1/100 dilution overnight at 4°C followed by a further incubation at room temperature for 1h with a goat anti-rabbit secondary antibody to (Alexa Fluor® 555) at 0.5 µg/ml. Acquisition of the green (wild-type), red (antibody staining) and far-red (knockout) channels was performed. Representative grayscale images of the red channel are shown. Wild-type and knockout cells are outlined with yellow and magenta dashed line, respectively. Schematic representation of the mosaic strategy used is shown on the bottom-right panel. Image was acquired with a Zeiss(LSM-880).

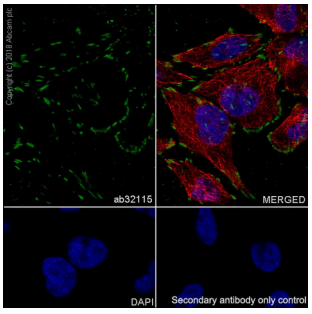
This data was provided by YCharOS Inc., an open science company with the mission of characterizing commercially available antibody reagents for all human proteins. Abcam and YCharOS are working together to help address the reproducibility crisis by enabling the life science community to better evaluate commercially available antibodies.



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

Paxillin Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-Paxillin antibody

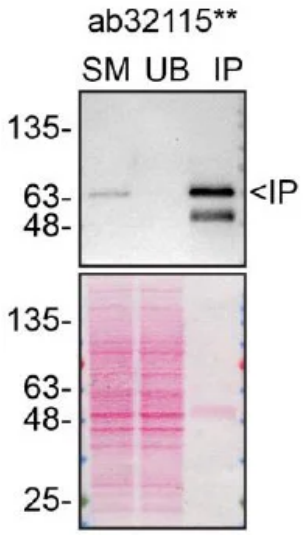
Immunohistochemical analysis of Paxillin expression in paraffin-embedded human colon carcinoma using 1/100 ab32115.



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

Paxillin Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Paxillin antibody

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.



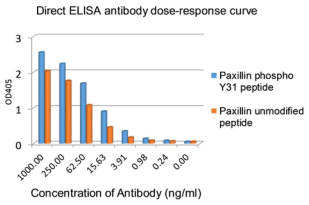
Immunoprecipitation - Anti-Paxillin antibody [E228] (ab32115)

Immunoprecipitation of PXN in A-431 cells. Lysates were prepared and immunoprecipitation was performed using 16.7 µg of ab32115 pre-coupled to Protein A beads. Samples were then washed and processed for western blot.

This data was provided by YCharOS Inc., an open science company with the mission of characterizing commercially available antibody reagents for all human proteins. Abcam and YCharOS are working together to help address the reproducibility crisis by enabling the life science community to better evaluate commercially available antibodies.
All lanes:
Immunoprecipitation - Anti-Paxillin antibody [E228] (ab32115) at 16.7 µg

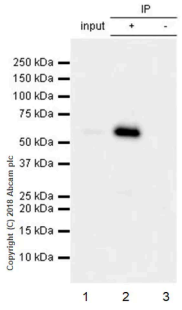
All lanes:
A-431 cell Lysates

Observed band size: 64 kDa



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

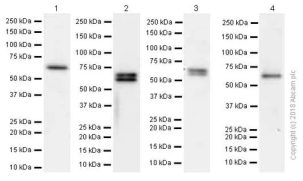
Direct ELISA antigen dose-response curve using ab32115 at 0-1000 ng/mL. Antigen (human Paxillin phospho Y31 peptide/ unmodified peptide) concentration of 1000 ng/mL. An alkaline phosphatase-conjugated goat anti-rabbit IgG (H+L) (1/2500) was used as the secondary antibody.



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.
All lanes:
Immunoprecipitation - Anti-Paxillin antibody [E228] (ab32115)

Predicted band size: 65 kDa



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

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

Lane 1:
HEK-293 (Human embryonic kidney) whole cell lysates at 15 µg

Lane 2:
NIH/3T3 (Mouse embryonic fibroblast) whole cell lysates at 15 µg

Lane 3:
C2C12 (Mouse myoblasts myoblast) whole cell lysates at 15 µg

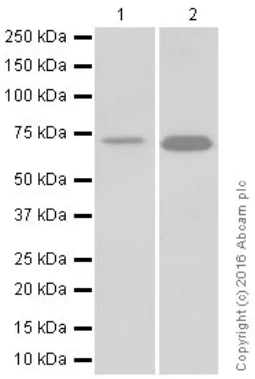
Lane 4:
Rat-1 (Rat embryonic fibroblast) whole cell lysates at 15 µg

Secondary

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

Predicted band size: 65 kDa

Observed band size: 60 kDa, 64 kDa



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

Paxillin Western blot staining using rabbit Anti-Paxillin antibody

Blocking and diluting buffer and concentration: 5% NFDM/TBST.
All lanes:
Western blot - Anti-Paxillin antibody [E228] (ab32115) at 1/1000 dilution

Lane 1:
HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysates at 15 µg

Lane 2:
HEK-293 (human embryonic kidney) whole cell lysates at 15 µg

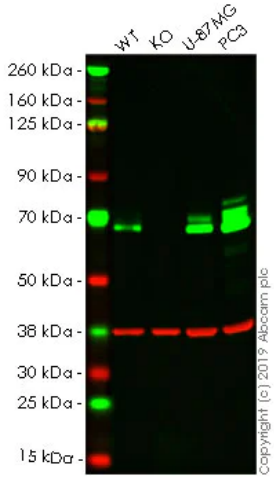
Secondary

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

Predicted band size: 65 kDa

Observed band size: 64 kDa

Exposure time: 3min



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

Paxillin Western blot staining using rabbit Anti-Paxillin antibody

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 A-431 cells as signal was lost in PXN knockout cell line ab261892 (knockout cell lysate ab261701). 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.

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

Lane 1:

Wild-type A431 whole cell lysate at 20 µg

Lane 2:
PXN knockout A431 whole cell lysate at 20 µg

Lane 2:
Western blot - Human PXN (Paxillin) knockout A-431 cell line (ab261892)

Lane 3:
U-87 MG whole cell lysate at 20 µg

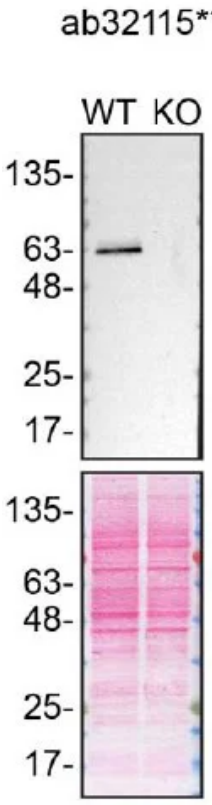
Lane 4:
PC-3 whole cell lysate at 20 µg

Performed under reducing conditions.

Predicted band size: 65 kDa

Observed band size: 65 kDa

Exposure time: 10s



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

Paxillin Western blot staining using rabbit Anti-Paxillin antibody

ab32115 was shown to react with PXN in wild-type A-431 cells in Western blot with loss of signal observed in PXN knockout cell line ab261892. Wild-type A-431 and PXN knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 5% milk in TBST for 1 hr before incubation with ab32115 overnight at 4 °C at a 1/10000 dilution. Blots were incubated with secondary antibodies at 0.2 µg/mL before imaging.

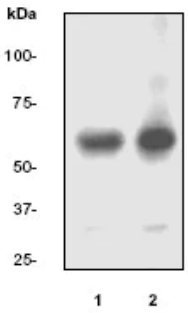
This data was provided by YCharOS Inc., an open science company with the mission of characterizing commercially available antibody reagents for all human proteins. Abcam and YCharOS are working together to help address the reproducibility crisis by enabling the life science community to better evaluate commercially available antibodies.
All lanes:
Western blot - Anti-Paxillin antibody [E228] (ab32115) at 1/10000 dilution

Lane 1:
Wild-type A-431 lysate at 30 µg

Lane 2:
PXN knock-out A-431 lysate at 30 µg

Lane 2:
Western blot - Human PXN (Paxillin) knockout A-431 cell line (ab261892)

Observed band size: 63 kDa



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

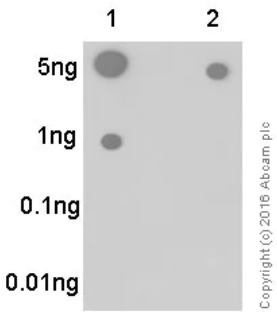
All lanes:
Western blot - Anti-Paxillin antibody [E228] (ab32115) at 1/10000 dilution

Lane 1:
untreated NIH 3T3 cell lysate

Lane 2:
PDGF treated NIH 3T3 cell lysate

Predicted band size: 65 kDa

Observed band size: 64 kDa



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.

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.