

### Anti-Paxillin antibody [E228] ab32115

KO 評価済 リコンビナント RabMAb

12 References 画像数 14

#### 製品の概要

製品名	Anti-Paxillin antibody [E228]
製品の詳細	Rabbit monoclonal [E228] to Paxillin
由来種	Rabbit
特異性	The mouse and rat recommendation is based on the WB results. We do not guarantee IHC-P for mouse and rat.
アプリケーション	<b>適用あり:</b> WB, ICC/IF, IHC-P, IP, Flow Cyt (Intra), Dot blot, ELISA
種交差性	<b>交差種:</b> Mouse, Rat, Human
免疫原	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
ポジティブ・コントロール	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.
特記事項	<p>This product is a recombinant monoclonal antibody, which offers several advantages including:</p> <ul style="list-style-type: none"> <li>- High batch-to-batch consistency and reproducibility</li> <li>- Improved sensitivity and specificity</li> <li>- Long-term security of supply</li> <li>- Animal-free production</li> </ul> <p>For more information <a href="#">see here</a>.</p> <p>Our RabMAb<sup>®</sup> technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to <a href="#">RabMAb<sup>®</sup> patents</a>.</p>

#### 製品の特性

製品の状態	Liquid
保存方法	Shipped at 4°C. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles.
バッファー	<p>pH: 7.20</p> <p>Preservative: 0.01% Sodium azide</p> <p>Constituents: 40% Glycerol (glycerin, glycerine), PBS, 0.05% BSA</p>
精製度	Protein A purified
ポリ/モノ	モノクローナル

クローン名	E228
アイソタイプ	IgG

#### アプリケーション

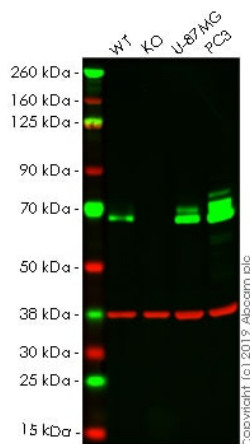
**The Abpromise guarantee**      **Abpromise保証は、**次のテスト済みアプリケーションにおけるab32115の使用に適用されます  
アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
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 <b><u>IHC antigen retrieval protocols.</u></b>  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.

#### ターゲット情報

機能	Cytoskeletal protein involved in actin-membrane attachment at sites of cell adhesion to the extracellular matrix (focal adhesion).
配列類似性	Belongs to the paxillin family. Contains 4 LIM zinc-binding domains.
翻訳後修飾	Phosphorylated on tyrosine residues during integrin-mediated cell adhesion, embryonic development, fibroblast transformation and following stimulation of cells by mitogens.
細胞内局在	Cytoplasm > cytoskeleton. Cell junction > focal adhesion.

#### 画像



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.

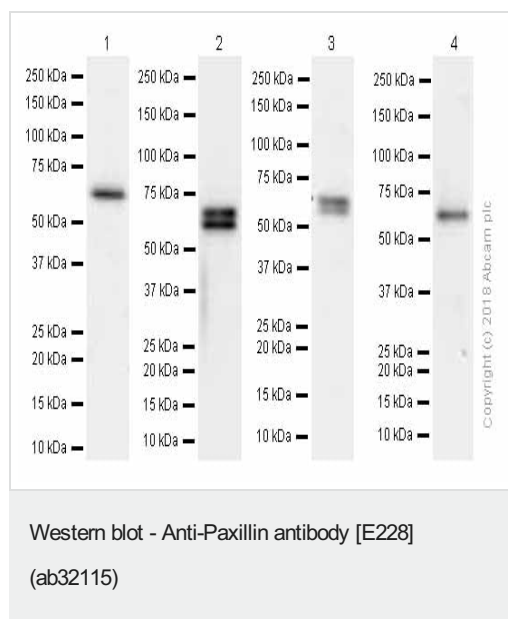
**Predicted band size:** 64 kDa

**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.



**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.

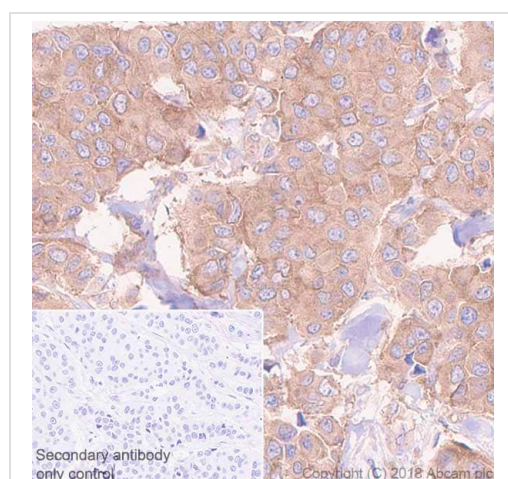
### Secondary

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

**Predicted band size:** 64 kDa

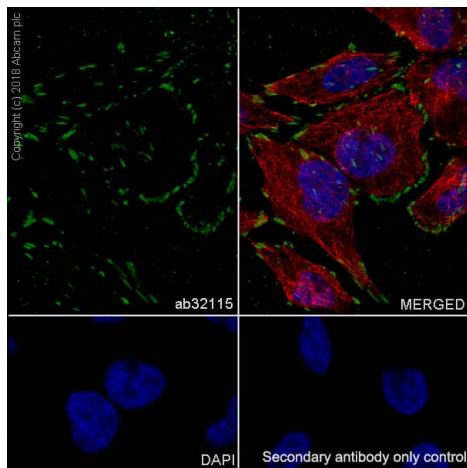
**Observed band size:** 60,64 kDa

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

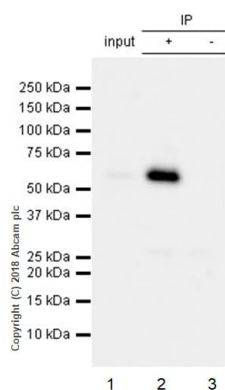


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Paxillin antibody [E228] (ab32115)

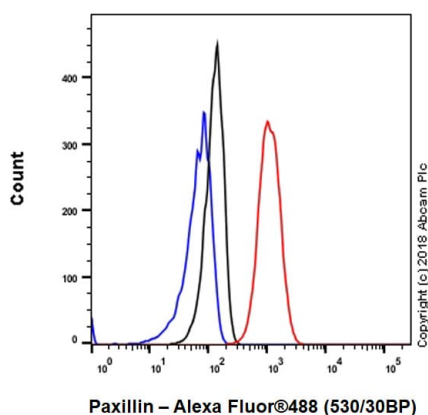
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Human breast carcinoma tissue sections labeling Paxillin with Purified ab32115 at 1:50 dilution (2.34 µg/ml). Heat mediated antigen retrieval was performed using [ab93684](#) (Tris/EDTA buffer, pH 9.0). ImmunoHistoProbe 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)



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

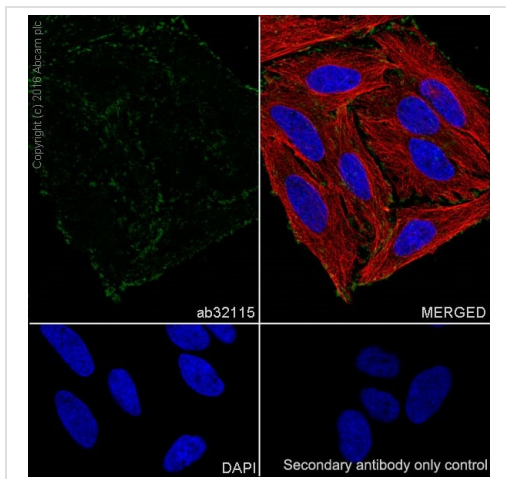


Flow Cytometry (Intracellular) - Anti-Paxillin antibody [E228] (ab32115)

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.

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.

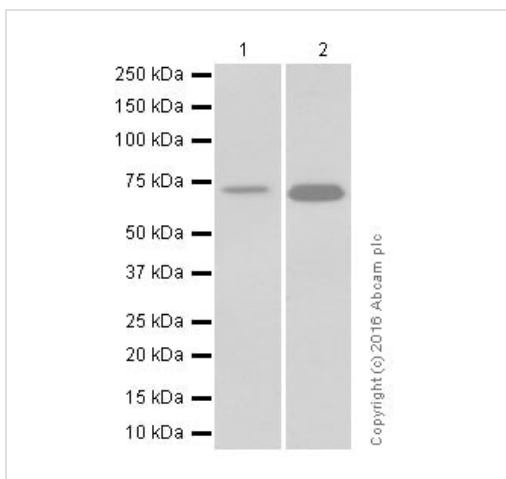
Intracellular Flow Cytometry analysis of HeLa (Human cervix adenocarcinoma epithelial cell) cells labeling Paxillin with Purified ab32115 at 1/20 dilution (10µg/ml) (red). Cells were fixed with 4% Paraformaldehyde. A Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) secondary antibody was used at 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

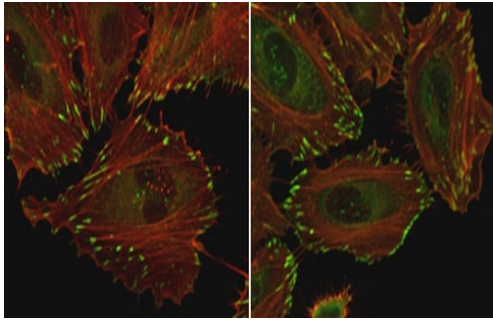
**All lanes** : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

**Predicted band size:** 64 kDa

**Observed band size:** 64 kDa

**Exposure time:** 3 minutes

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

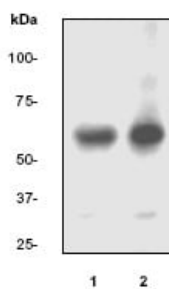


Immunocytochemistry/ Immunofluorescence - Anti-Paxillin antibody [E228] (ab32115)

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)

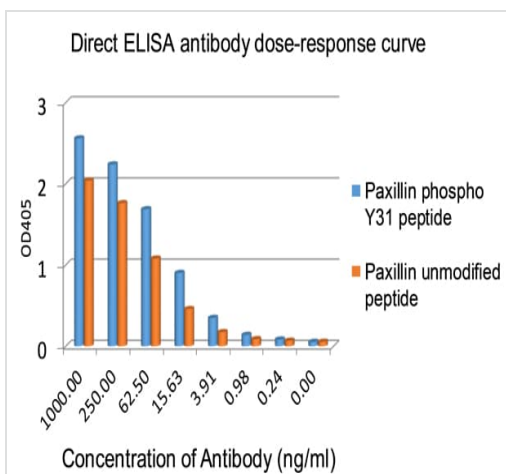
**All lanes :** Anti-Paxillin antibody [E228] (ab32115) at 1/10000 dilution

**Lane 1 :** untreated NIH 3T3 cell lysate

**Lane 2 :** PDGF treated NIH 3T3 cell lysate

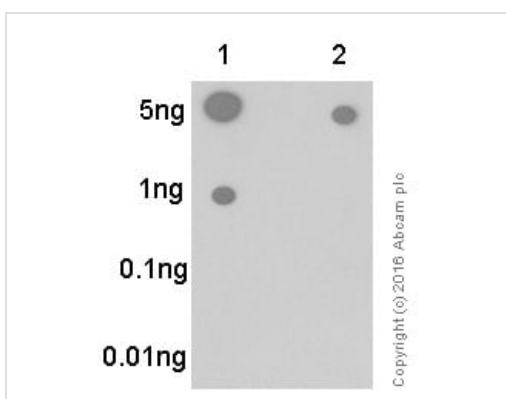
**Predicted band size:** 64 kDa

**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.

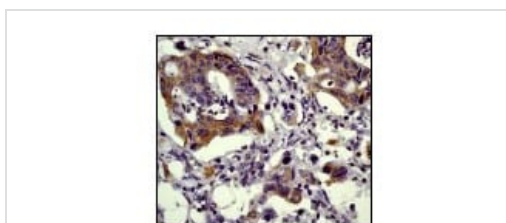


Dot Blot - Anti-Paxillin antibody [E228] (ab32115)

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.



### Why choose a recombinant antibody?



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Recombinant technology



**Success from the first experiment**  
Confirmed specificity



**Ethical standards compliant**  
Animal-free production

Anti-Paxillin antibody [E228] (ab32115)

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