Anti-Paxillin antibody [Y113] ab32084

Overview

Product name  Anti-Paxillin antibody [Y113]
Description  Rabbit monoclonal [Y113] to Paxillin
Host species  Rabbit
Specificity  ab32084 recognises Paxillin alpha, beta and gamma isoforms.
Tested applications  Suitable for: WB, IHC-P, ICC/IF, Flow Cyt, IP
Species reactivity  Reacts with: Mouse, Rat, Cow, Dog, Human
Immunogen  Synthetic peptide within Human Paxillin aa 1-100 (N terminal). The exact sequence is proprietary.
Positive control  WB: HeLa cell lysate; RAW264.7 whole cell lysate. IHC-P: Human breast carcinoma; Normal ovary tissue; Ovarian carcinoma tissue; Transitional cell carcinoma of kidney tissue. ICC/IF: 3T3 fibroblasts; Mouse embryonic fibroblasts; MEF1 cells treated with (S)-(−)-Blebbistatin; bovine kidney cells. Flow Cyt: HeLa cells.

General notes

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

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. Avoid freeze / thaw cycle.
Dissociation constant ($K_D$)  $K_D = 4.17 \times 10^{-10}$ M
Storage buffer  pH: 7.20
Preservative: 0.01% Sodium azide
Constituents: 49% PBS, 50% Glycerol, 0.05% BSA

**Clonality**
Monoclonal

**Clone number**
Y113

**Isotype**
IgG

**Applications**

Our **Abpromise guarantee** covers the use of **ab32084** in the following tested applications.

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

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<td>Use at an assay dependent concentration.</td>
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<td>Flow Cyt</td>
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<td>Use at an assay dependent concentration.</td>
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<tr>
<td>IP</td>
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<td>1/200.</td>
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**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**
Image: Courtesy of Dr. Shaohua Li, UMDNJ-Robert Wood Johnson Medical School

Sample: mouse embryonic fibroblasts

Preparation:

Fix in 3% PFA in PBS for 30 min at RT

Primary antibody: Rabbit anti-paxillin Y113 (ab32084), 1:100

Secondary antibody: Goat polyclonal Secondary Antibody to Rabbit IgG - H&L (Alexa Fluor® 488) pre-adsorbed (ab150081), 1:200

Rhodamine-phalloidin, 1:100

Nuclei were counterstained with DAPI

Shear-induced Cell Remodeling.

3T3 fibroblasts are shown under the indicated cation and shear conditions. The shear direction in each image is indicated by a white arrow. Images show paxillin in green (ab32084), the actin cytoskeleton in red, and the nucleus (DNA) in blue. The approximate pre-shear cell area is indicated by white dashed lines as determined from the focal adhesions that remained on the substrate, which are indicated by open arrowheads. The bottom left image was contrast-enhanced 2-fold to better visualize the focal adhesions that remained on the substrate. Inset images are shown from regions outlined in white.
Anti-Paxillin antibody [Y113] (ab32084) at 1/10000 dilution + HeLa cell lysate.

**Predicted band size:** 68 kDa  
**Observed band size:** 60 kDa  
*why is the actual band size different from the predicted?*

ab32084 staining paxillin in MEF1 cells treated with (+/-)-blebbistatin (ab120425), by ICC/IF. Decreased membrane expression of paxillin correlates with increased concentration of (+/-)-blebbistatin, as described in literature.

The cells were incubated at 37°C for 1h in media containing different concentrations of ab120425 ((+/-)-blebbistatin) in DMSO, fixed with 4% formaldehyde for 10 minutes at room temperature and blocked with PBS containing 10% goat serum, 0.3 M glycine, 1% BSA and 0.1% tween for 2h at room temperature. Staining of the treated cells with ab32084 (1/100 dilution) was performed overnight at 4°C in PBS containing 1% BSA and 0.1% tween. A DyLight 488 goat anti-rabbit polyclonal antibody (ab96899) at 1/250 dilution was used as the secondary antibody. Nuclei were counterstained with DAPI and are shown in blue.

ab32084 showing positive staining in Ovarian carcinoma tissue.
Flow cytometry analysis of HeLa (human cervix adenocarcinoma) cells labeling with purified ab32084 at 1/100 dilution (10ug/ml) (Red). Cells were fixed with 4% paraformaldehyde and permeabilised with 90% methanol. A Goat anti rabbit IgG (Alexa Fluor® 488) (ab150077) (1/2000 dilution) was used as the secondary antibody. Rabbit monoclonal IgG (Black) (ab172730) was used as a isotype control. Cell without incubation with primary antibody and secondary antibody (Blue) were used as unlabeled control.

ab32084 staining paxillin in MEF1 cells treated with (S)-(−)-Blebbistatin (ab120491), by ICC/IF. Decreased membrane expression of paxillin correlates with increased concentration of (S)-(−)-Blebbistatin, as described in literature. The cells were incubated at 37°C for 2h in media containing different concentrations of ab120491 ( (S)-(−)-Blebbistatin ) in DMSO, fixed with 4% formaldehyde for 10 minutes at room temperature and blocked with PBS containing 10% goat serum, 0.3 M glycine, 1% BSA and 0.1% tween for 2h at room temperature. Staining of the treated cells with ab32084 (1/100 dilution) was performed overnight at 4°C in PBS containing 1% BSA and 0.1% tween. A DyLight 488 goat anti-rabbit polyclonal antibody (ab96899) at 1/250 dilution was used as the secondary antibody. Nuclei were counterstained with DAPI and are shown in blue.

ab32084 showing positive staining in Normal ovary tissue.
Western blot - Anti-Paxillin antibody [Y113] (ab32084)

This image is courtesy of an Abreview submitted by Dr. Alban Gaultier

**Primary antibody**
Anti-Paxillin antibody [Y113] (ab32084) at 1/5000 dilution + Mouse RAW264.7 whole cell lysate at 20 µg

**Secondary**
An HRP Donkey anti-rabbit IgG polyclonal at 1/10000 dilution

**Predicted band size:** 68 kDa

**Blocking step:** 5% Milk for 1 hour at 20°C.

**Gel:** SDS-PAGE

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Paxillin antibody [Y113] (ab32084)

*Immunohistochemistry analysis of bovine kidney cells, staining Paxillin with ab32084. Cells were fixed with paraformaldehyde, permeabilized with 1% Triton X-100 and blocked with 5% BSA for 1 hour. Samples were incubated with primary antibody (1/2500 in 5% BSA) for 1 hour at 25°C. An undiluted AlexaFluor®488-conjugated goat anti-rabbit polyclonal IgG was used as the secondary antibody.*

Immunocytochemistry/ Immunofluorescence - Anti-Paxillin antibody [Y113] (ab32084)

*This image is courtesy of an anonymous Abreview.*
Equilibrium disassociation constant ($K_D$)

Learn more about $K_D$

Click here to learn more about $K_D$

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