abcam

Product datasheet

Anti-Paxillin (phospho S126) antibody ab24402

1 References 4 Images

Overview

Product name	Anti-Paxillin (phospho S126) antibody
Description	Rabbit polyclonal to Paxillin (phospho S126)
Host species	Rabbit
Tested applications	**Suitable for:** IHC-P, ICC/IF, WB
Species reactivity	**Reacts with:** Mouse, Human **Predicted to work with:** Chicken
Immunogen	Synthetic peptide corresponding to Human Paxillin (phospho S126).
Positive control	WB: RAW 264.7 cells treated with LPS. A549. HepG2 IHC-P: human breast carcinoma ICC/IF: NIH/3T3
General notes	The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets your needs before purchasing. If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, along with publications, customer reviews and Q&As

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 long term.
Storage buffer	pH: 7.30 Preservative: 0.05% Sodium azide Constituents: PBS, 50% Glycerol (glycerin, glycerine), 0.1% BSA
Purity	Immunogen affinity purified
Purification notes	Purified from rabbit serum by sequential epitope-specific chromatography. The antibody has been negatively preadsorbed using a non-phosphopeptide corresponding to the site of phosphorylation to remove antibody that is reactive with non-phosphorylated paxillin. The final product is generated by affinity chromatography using a paxillin-derived peptide that is phosphorylated at serine 126.

Clonality	Polyclonal
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab24402 in the following tested applications.

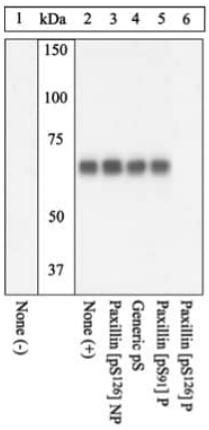
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
IHC-P		1/20. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
ICC/IF		1/250.
WB		1/1000. Detects a band of approximately 68 kDa.

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 (phospho S126) antibody (ab24402)

All lanes : Anti-Paxillin (phospho S126) antibody (ab24402) at 1/1000 dilution

Lane 1 : Lysate from untreated RAW 264.7 cells

Lane 2 : Lysate from RAW 264.7 cells treated with 1 µg/mL LPS for 60 minutes

Lane 3 : Lysate from RAW 264.7 cells treated with 1 µg/mL LPS for 60 minutes with non-phosphorylated peptide corresponding to the immunogen

Lane 4 : Lysate from RAW 264.7 cells treated with 1 µg/mL LPS for 60 minutes with generic phosphoserine-containing peptide

Lane 5 : Lysate from RAW 264.7 cells treated with 1 µg/mL LPS for 60 minutes with phosphopeptide corresponding to Paxillin [pS^{91}]

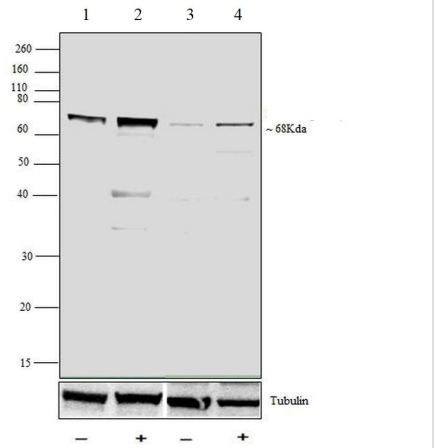
Lane 6 : Lysate from RAW 264.7 cells treated with 1 µg/mL LPS

for 60 minutes with phosphopeptide immunogen

Secondary
All lanes : Goat F(ab)$_2$ anti-rabbit IgG HRP conjugate.

Observed band size: 68 kDa

Lysates were resolved on a 10% polyacrylamide gel and transferred to PVDF. The membrane was blocked with a 5% milk-TBST buffer for one hour at room temperature, and then incubated with the Paxillin [pS^{126}] antibody for two hours at room temperature in a 1% milk-TBST buffer, following its prior incubation with or without peptides. Following incubation with goat F(ab')$_2$ anti-rabbit IgG HRP conjugate and bands were detected using the Pierce SuperSignal method. The data show that only the phosphopeptide corresponding to Paxillin [pS 126] blocks the antibody signal, thereby demonstrating the specificity of the antibody. No competition was seen following incubation with paxillin phosphopeptides to S^{130}, S^{178}, S^{380}, S^{455}, or S^{479} (not shown). The antibody was also shown to be specific using 293 cells transfected with wild-type EGFP-tagged human paxillin treated with EGF (not shown).



Western blot - Anti-Paxillin (phospho S126) antibody (ab24402)

All lanes : Anti-Paxillin (phospho S126) antibody (ab24402) at 1/1000 dilution

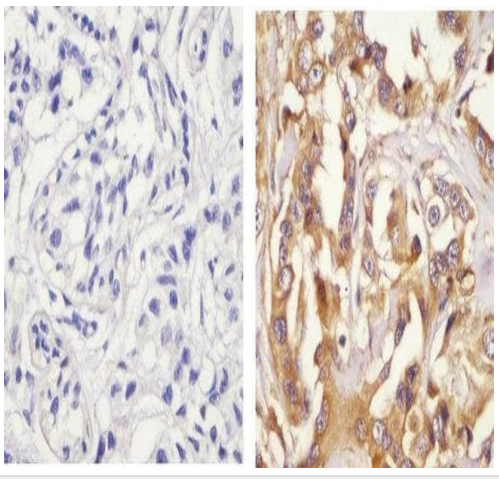
Lane 1 : A549 (Human lung carcinoma cell line) whole cell lysate
Lane 2 : A549 whole cell lysate treated with 0.1 ug/mL of HGF treatment for 10 minutes
Lane 3 : HepG2 (Human liver hepatocellular carcinoma cell line) whole cell lysate
Lane 4 : HepG2 whole cell lysate with 0.1 ug/mL of HGF treatment for 10 minutes

Lysates/proteins at 30 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG (H+L) Secondary Antibody, HRP conjugate at 1/1000 dilution

Observed band size: 68 kDa

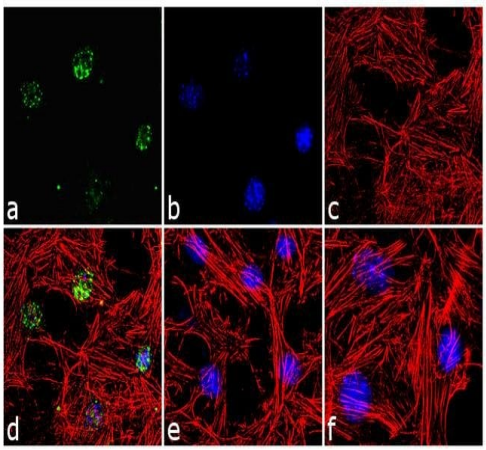
The membrane was probed with the relevant primary and secondary Antibody following blocking with 5 % skimmed milk. Chemiluminescent detection was performed using Pierce™ ECL Western Blotting Substrate



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Paxillin (phospho S126) antibody (ab24402)

Immunohistochemical analysis of human breast carcinoma labeling Paxillin (phospho S126) with ab24402 at 1/20 dilution (right) compared to a negative control without primary antibody (left).

To expose target proteins, antigen retrieval was performed using 10mM sodium citrate (pH 6.0), microwaved for 8-15 min. Following antigen retrieval, tissues were blocked in 3% H2O2-methanol for 15 min at room temperature, washed with ddH2O and PBS, and then probed with ab24402 diluted in 3% BSA-PBS at a dilution of 1/20 overnight at 4ºC in a humidified chamber. Tissues were washed extensively in PBST and detection was performed using an HRP-conjugated secondary antibody followed by colorimetric detection using a DAB kit. Tissues were counterstained with hematoxylin and dehydrated with ethanol and xylene to prep for mounting



Immunocytochemistry/ Immunofluorescence - Anti-Paxillin (phospho S126) antibody (ab24402)

Immunofluorescence analysis of 70% confluent log phase NIH/3T3 cells treated with 0.5 ug of PDGF for 10 minutes. The cells were fixed with 4% paraformaldehyde for 10 minutes, permeabilized with 0.1% Triton™ X-100 for 10 minutes, and blocked with 1% BSA for 1 hour at room temperature. Labeling Paxillin (phospho S126) with ab24402 at 1/250 dilution in 0.1% BSA and incubated for 3 hours at room temperature. Followed by Goat anti-Rabbit IgG (H+L) Superclonal™ Secondary Antibody, Alexa Fluor® 488 conjugate at a dilution of 1/2000 for 45 minutes at room temperature (Panel a: green). Nuclei (Panel b: blue) were stained with SlowFade® Gold Antifade Mountant with DAPI. F-actin (Panel c: red) was stained with Rhodamine Phalloidin at 1/300 dilution. Panel d is a merged image showing punctuated nuclear localization. Panel e is untreated cell with no signal. Panel f is a no primary antibody control. The images were captured at 60X magnification.

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