abcam

Product datasheet

Anti-Zyxin antibody [EPR4302] ab109316

Recombinant RabMAb

8 References 12 Images

Overview

Product name	Anti-Zyxin antibody [EPR4302]
Description	Rabbit monoclonal [EPR4302] to Zyxin
Host species	Rabbit
Tested applications	**Suitable for:** Flow Cyt (Intra), WB, IP, IHC-P, ICC/IF
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: HeLa, MCF7, Daudi and C2C12 cell lysates; mouse lung and testis, rat lung and testis tissue lysates. IHC-P: Human gastric carcinoma, mouse and rat kidney tissue IP: Mouse testis tissue lysate. Flow Cyt (intra): HeLa cells. ICC/IF: HeLa cells
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. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: 0.05% BSA, 40% Glycerol (glycerin, glycerine), 59% PBS
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR4302

Isotype IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab109316 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
Flow Cyt (Intra)		1/100. **ab172730** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
WB		1/20000. Detects a band of approximately 82 kDa (predicted molecular weight: 61 kDa).
IP		1/50.
IHC-P		1/1000. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol. See **IHC antigen retrieval protocols**. The immunostaining was performed on a Leica Biosystems BOND® RX instrument.
ICC/IF		1/500.

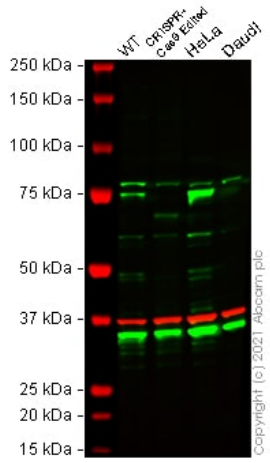
Target

Function Adhesion plaque protein. Binds alpha-actinin and the CRP protein. Important for targeting TES and ENA/VASP family members to focal adhesions and for the formation of actin-rich structures. May be a component of a signal transduction pathway that mediates adhesion-stimulated changes in gene expression.

Sequence similarities Belongs to the zyxin/ajuba family.
Contains 3 LIM zinc-binding domains.

Cellular localization Cytoplasm. Cytoplasm, cytoskeleton. Nucleus. Cell junction, focal adhesion. Associates with the actin cytoskeleton near the adhesion plaques. Enters the nucleus in the presence of HESX1.

Images



Western blot - Anti-Zyxin antibody [EPR4302] (ab109316)

All lanes : Anti-Zyxin antibody [EPR4302] (ab109316) at 1/20000 dilution

Lane 1 : Wild-type HEK-293T cell lysate
Lane 2 : ZYX CRISPR-Cas9 edited HEK-293T cell lysate
Lane 3 : HeLa cell lysate
Lane 4 : Daudi cell lysate

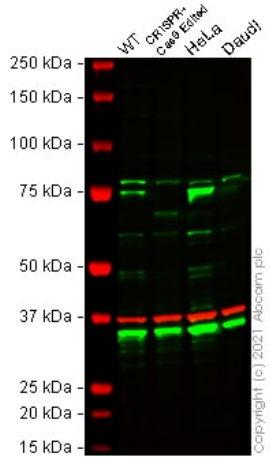
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 61 kDa
Observed band size: 75 kDa

False colour image of Western blot: Anti-Zyxin antibody [EPR4302] staining at 1/20000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (**ab8245**) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab109316 was shown to bind specifically to Zyxin. A band was observed at 75 kDa in wild-type HEK-293T cell lysates with no signal observed at this size in ZYX CRISPR-Cas9 edited cell line **ab266503** (CRISPR-Cas9 edited cell lysate **ab257809**). The band observed in the CRISPR-Cas9 edited lysate lane below 75 kDa is likely to represent a truncated form of Zyxin. This has not been investigated further and the functional properties of the gene product have not been determined. To generate this image, wild-type and ZYX CRISPR-Cas9 edited HEK-293T cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in fluorescent western blot (TBS-based) blocking solution before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed

(**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) at 1/20000 dilution.



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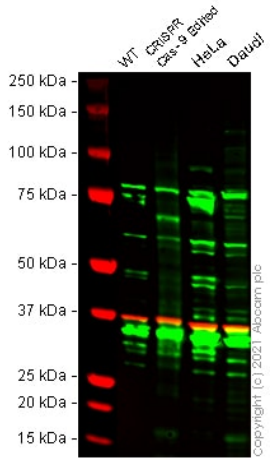

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Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

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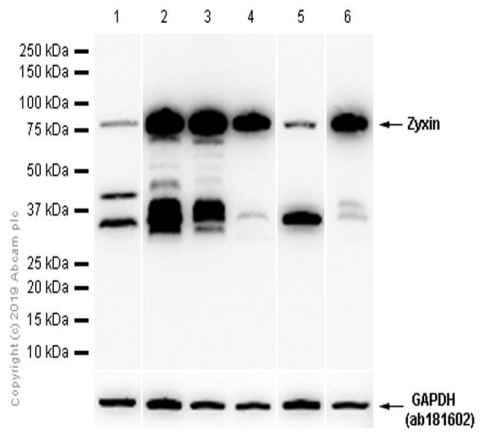


Western blot - Anti-Zyxin antibody [EPR4302] (ab109316)

All lanes : Anti-Zyxin antibody [EPR4302] (ab109316) at 1/20000 dilution (Purified)

Lane 1 : MCF7 (Human breast adenocarcinoma epithelial cell) whole cell lysates
Lane 2 : C2C12 (Mouse myoblasts myoblast) whole cell lysates
Lane 3 : Mouse lung lysates
Lane 4 : Mouse testis lysates
Lane 5 : Rat lung lysates
Lane 6 : Rat testis lysates

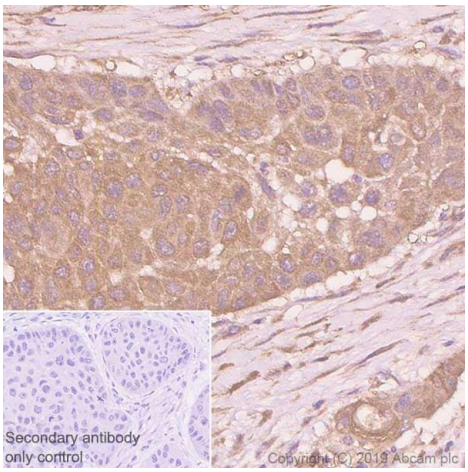
Lysates/proteins at 20 µg per lane.

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

Predicted band size: 61 kDa
Observed band size: 82 kDa

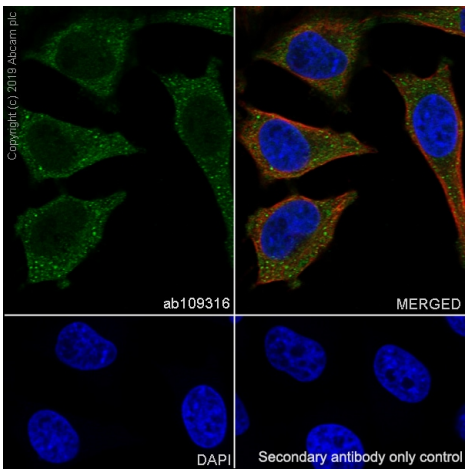
Blocking/Diluting buffer: 5% NFDM/TBST

We are unsure about the extra bands. They might be the cleavage fragments as what are described in PMID:17572661



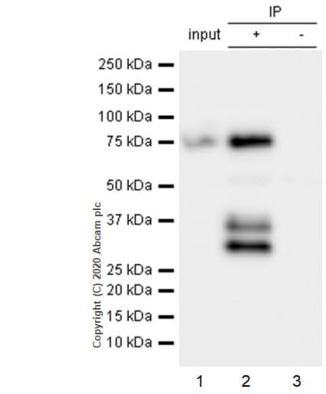
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Zyxin antibody [EPR4302] (ab109316)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human gastric carcinoma tissue sections labeling Zyxin with purified ab109316 at 1/1000 dilution (0.94 µg/mL). Heat mediated antigen retrieval using Bond™ Epitope Retrieval Solution 2 (pH 9.0). Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**) was used as the secondary antibody. Negative control: PBS instead of the primary antibody. Hematoxylin was used as a counterstain.



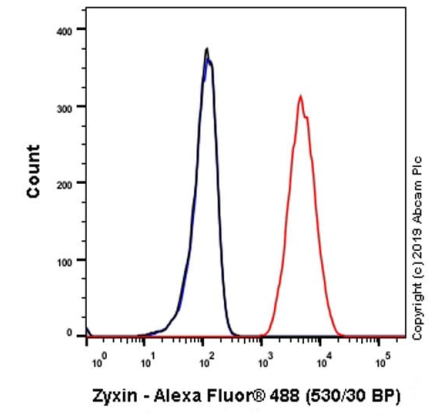
Immunocytochemistry/ Immunofluorescence - Anti-Zyxin antibody [EPR4302] (ab109316)

Immunocytochemistry/ Immunofluorescence analysis of HeLa (Human cervix adenocarcinoma epithelial cell) cells labeling Zyxin with Purified ab109316 at 1/500 dilution (1.87 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 (blue) was used as nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.



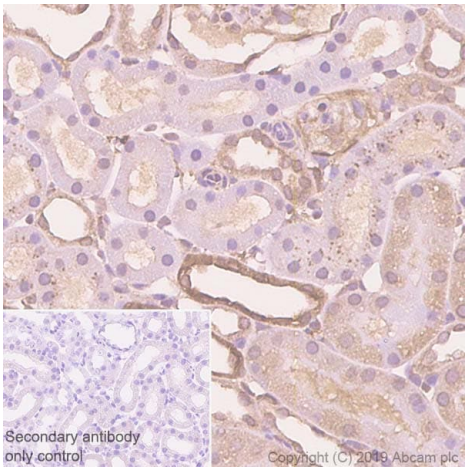
Immunoprecipitation - Anti-Zyxin antibody [EPR4302] (ab109316)

Purified ab109316 at 1/50 dilution (2ug) immunoprecipitating Zyxin in Mouse testis lysate.
Lane 1 (input): Mouse testis lysate (10µg)
Lane 2 (+): ab109316 + Mouse testis lysate.
Lane 3 (-): Rabbit monoclonal IgG (**ab172730**) instead of ab109316 in mouse testis lysate.
VeriBlot for IP Detection Reagent (HRP) (**ab131366**) (1/5000) was used for Western blotting.
Blocking Buffer and concentration: 5% NFDM/TBST.
Diluting buffer and concentration: 5% NFDM/TBST.
Observed band size: 82 kDa
We are unsure about the extra bands. They might be the cleavage fragments as what are described in PMID:17572661



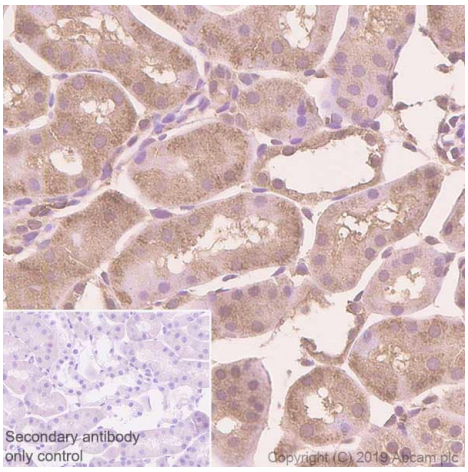
Flow Cytometry (Intracellular) - Anti-Zyxin antibody [EPR4302] (ab109316)

Intracellular Flow Cytometry analysis of HeLa (Human cervix adenocarcinoma epithelial cell) cells labeling Zyxin with Purified ab109316 at 1/100 dilution (10 µg/mL) (Red). Cells were fixed with 4% Paraformaldehyde and permeabilised with 90% Methanol. 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).



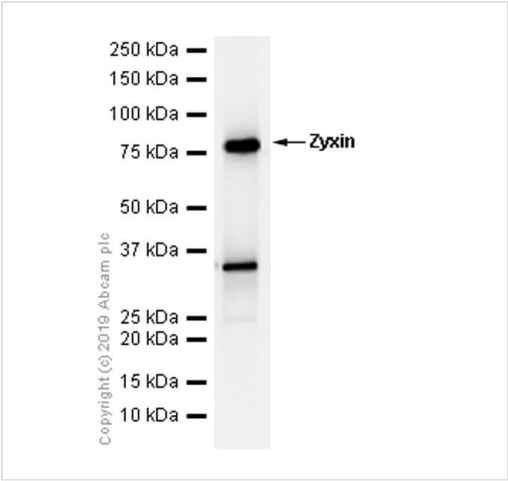
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Zyxin antibody [EPR4302] (ab109316)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of rat kidney tissue sections labeling Zyxin with purified ab109316 at 1/1000 dilution (0.94 µg/mL). Heat mediated antigen retrieval using Bond™ Epitope Retrieval Solution 2 (pH 9.0). Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**) was used as the secondary antibody. Negative control: PBS instead of the primary antibody. Hematoxylin was used as a counterstain.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Zyxin antibody [EPR4302] (ab109316)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of mouse kidney tissue sections labeling Zyxin with purified ab109316 at 1/1000 dilution (0.94 µg/mL). Heat mediated antigen retrieval using Bond™ Epitope Retrieval Solution 2 (pH 9.0). Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**) was used as the secondary antibody. Negative control: PBS instead of the primary antibody. Hematoxylin was used as a counterstain.



Western blot - Anti-Zyxin antibody [EPR4302] (ab109316)

Anti-Zyxin antibody [EPR4302] (ab109316) at 1/20000 dilution (Purified) + HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysates at 15 µg

Secondary

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

Predicted band size: 61 kDa

Observed band size: 82 kDa

Blocking/Diluting buffer: 5% NFDM/TBST

We are unsure about the extra bands. They might be the cleavage fragments as what are described in PMID:17572661

Anti-Zyxin antibody [EPR4302] (ab109316)

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