# abcam

### Product datasheet

## Anti-Zyxin antibody [EPR4302] ab109316

יעלצעבע RabMAb

8 References 画像数 12

#### 製品の概要

製品名 Anti-Zyxin antibody [EPR4302]

製品の詳細 Rabbit monoclonal [EPR4302] to Zyxin

由来種 Rabbit

アプリケーション 適用あり: Flow Cyt (Intra), WB, IP, IHC-P, ICC/IF

種交差性 交差種: 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, 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

特記事項 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**.

#### 製品の特性

製品の状態 Liquid

保存方法 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.

バッファー pH: 7.2

Preservative: 0.01% Sodium azide

Constituents: 0.05% BSA, 40% Glycerol (glycerin, glycerine), 59% PBS

精製度 Protein A purified

ポリモノ モノクローナル

クローン名 EPR4302

## アプリケーション

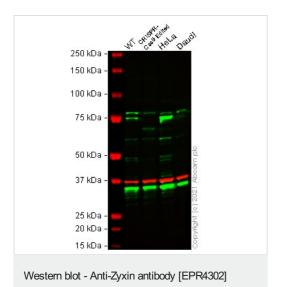
**The Abpromise guarantee** <u>Abpromise保証は、</u>次のテスト済みアプリケーションにおけるab109316の使用に適用されます アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
Flow Cyt (Intra)		1/100. <b>ab172730</b> - Rabbit monoclonal lgG, 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 <b>IHC antigen retrieval protocols</b> .  The immunostaining was performed on a Leica Biosystems BOND <sup>®</sup> RX instrument.
ICC/IF		1/500.

## ターゲット情報

機能	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.
配列類似性	Belongs to the zyxin/ajuba family.  Contains 3 LIM zinc-binding domains.
細胞内局在	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.

## 画像



(ab109316)

dilution

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

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

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

250 kDa - 150 kDa - 75 kDa - 20 kDa - 20 kDa - 15 kDa - 1

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

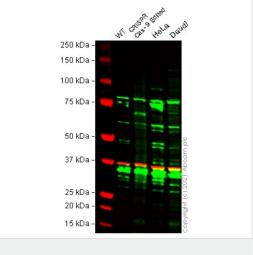
Lane 4 : Daudi cell lysate

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 lgG H&L (IRDye® 800CW) preabsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (ab216776) at 1/20000 dilution.



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

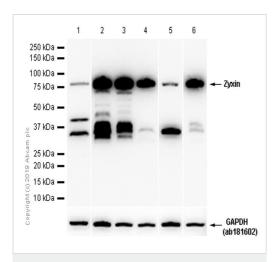
Lane 4 : Daudi cell lysate

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 ab266504 (CRISPR-Cas9 edited cell lysate ab257810). 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.



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) (<u>ab97051</u>) at 1/20000 dilution

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

Blocking/Diluting buffer: 5% NFDM/TBST

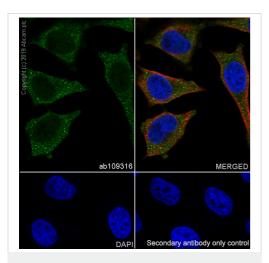
We are unsure about the extra bands. They might be the cleavage fragments as what are described in PMID:17572661

Secondary antibody
only control

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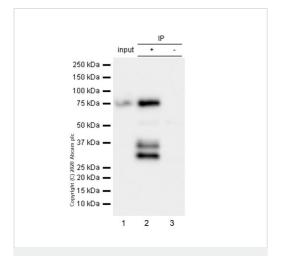
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Zyxin antibody
[EPR4302] (ab109316)

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.

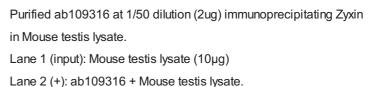


Immunocytochemistry/ Immunofluorescence - Anti-Zyxin antibody [EPR4302] (ab109316)

Immunocytochemistry/ Immunofluorescence analysis of HeLa (Human cervix adenocarcinoma epithelial cell) cells labeling Zyxin with Purified ab109316 at 1/500 dilution (1.87  $\mu$ g/mL). Cells were fixed in 4% Paraformaldehyde and permeabilized with 0.1% tritonX-100. Cells were counterstained with <u>ab195889</u> Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) 1/200 (2.5  $\mu$ g/mL). Goat anti rabbit lgG (Alexa Fluor® 488, <u>ab150077</u>) was used as the secondary antibody at 1/1000 (2  $\mu$ g/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)



Lane 3 (-): Rabbit monoclonal lgG (ab172730) instead of

ab109316 in mouse testis lysate.

VeriBlot for IP Detection Reagent (HRP) (ab131366) (1/5000) was

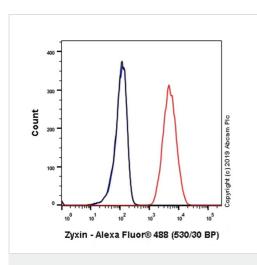
Blocking Buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM/TBST.

Observed band size: 82 kDa

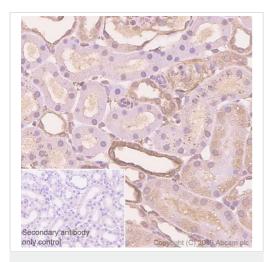
used for Western blotting.

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



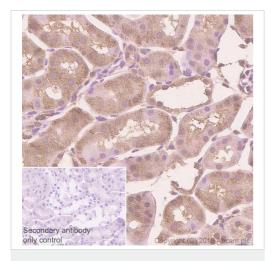
Flow Cytometry (Intracellular) - Anti-Zyxin antibody [EPR4302] (ab109316)

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 lgG (Alexa Fluor<sup>®</sup> 488, **ab150077**) secondary antibody was used at 1/2000. Isotype control - Rabbit monoclonal lgG (Black). Unlabeled control - Cell without incubation with primary antibody and secondary antibody (Blue).



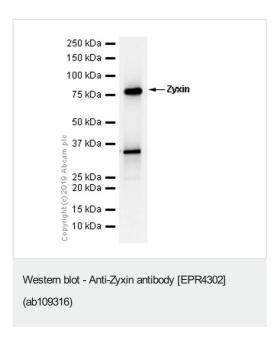
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Zyxin antibody
[EPR4302] (ab109316)

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 paraffinembedded 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.



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

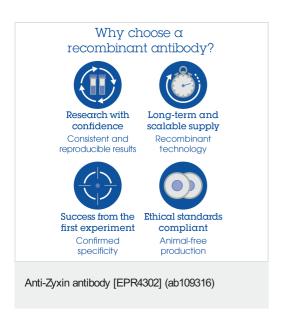
#### **Secondary**

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



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