abcam

Product datasheet

Anti-Zyxin antibody [EPR4302] - BSA and Azide free ab238430

יעלאעבע RabMAb

画像数 11

製品の概要

製品名 Anti-Zyxin antibody [EPR4302] - BSA and Azide free

製品の詳細 Rabbit monoclonal [EPR4302] to Zyxin - BSA and Azide free

由来種 Rabbit

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

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 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 ab238430 is the carrier-free version of ab109316.

> Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency.

This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cellbased assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.

Use our conjugation kits for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold.

This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar[®] is a trademark of Fluidigm Canada Inc.

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. Do Not Freeze.

バッファー pH: 7.2

Constituent: PBS

キャリア・フリー はい

精製度 Protein A purified

ポリ/モノ モノクローナル

クローン名 EPR4302

アイソタイプ IgG

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ab238430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) | | Use at an assay dependent concentration. |
| WB | | Use at an assay dependent concentration. Detects a band of approximately 82 kDa (predicted molecular weight: 61 kDa). |
| IHC-P | | Use at an assay dependent concentration. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. See IHC antigen retrieval protocols. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. |
| ICC/IF | | Use at an assay dependent concentration. |
| IP | | Use at an assay dependent concentration. |

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.

 $\label{thm:may-be} \mbox{May be a component of a signal transduction pathway that mediates adhesion-stimulated}$

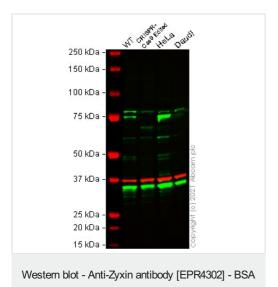
changes in gene expression.

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.



and Azide free (ab238430)

All lanes : Anti-Zyxin antibody [EPR4302] (<u>ab109316</u>) 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

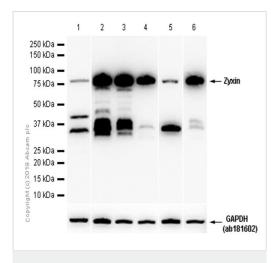
Lane 4 : Daudi cell lysate

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.



Western blot - Anti-Zyxin antibody [EPR4302] - BSA and Azide free (ab238430)

All lanes : Anti-Zyxin antibody [EPR4302] (<u>ab109316</u>) 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 5 : Rat lung lysates

Lane 6: Rat testis lysates

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/20000 dilution

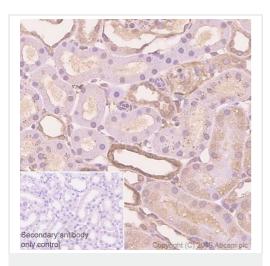
Predicted band size: 61 kDa **Observed band size:** 82 kDa

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

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab109316).

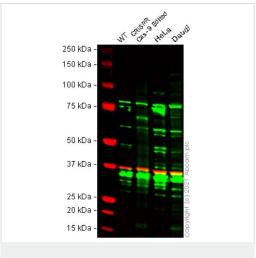
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of rat kidney tissue sections labeling Zyxin with Purified <u>ab109316</u> at 1/1000 dilution (0.94 µg/mL). Heat mediated antigen retrieval using Bond™ Epitope Retrieval Solution 2 (pH 9.0). Rabbit specific IHC polymer detection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

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab109316</u>).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Zyxin antibody

[EPR4302] - BSA and Azide free (ab238430)



Western blot - Anti-Zyxin antibody [EPR4302] - BSA and Azide free (ab238430)

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

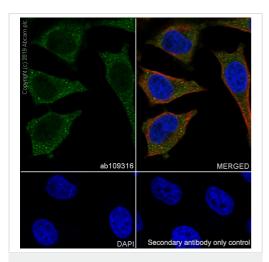
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

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

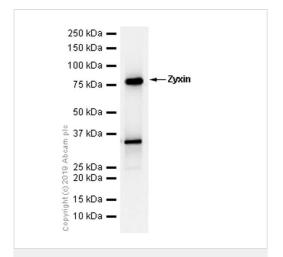
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab109316</u>).

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.



Immunocytochemistry/ Immunofluorescence - Anti-Zyxin antibody [EPR4302] - BSA and Azide free (ab238430)

Immunocytochemistry/ Immunofluorescence analysis of HeLa (Human cervix adenocarcinoma epithelial cell) cells labeling Zyxin with Purified <u>ab109316</u> 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 <u>ab195889</u> Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) 1/200 (2.5 μg/mL). Goat anti rabbit lgG (Alexa Fluor® 488, <u>ab150077</u>) 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. This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab109316</u>).



Western blot - Anti-Zyxin antibody [EPR4302] - BSA and Azide free (ab238430)

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

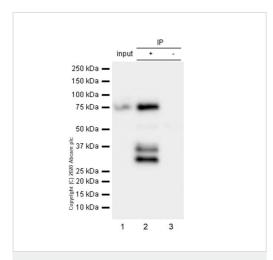
Secondary

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

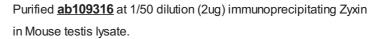
Predicted band size: 61 kDa
Observed band size: 82 kDa

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

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab109316</u>).



Immunoprecipitation - Anti-Zyxin antibody
[EPR4302] - BSA and Azide free (ab238430)



Lane 1 (input): Mouse testis lysate (10µg)

Lane 2 (+): <u>ab109316</u> + Mouse testis lysate.

Lane 3 (-): Rabbit monoclonal IgG (<u>ab172730</u>) instead of <u>ab109316</u> in mouse testis lysate.

VeriBlot for IP Detection Reagent (HRP) (<u>ab131366</u>) (1/5000) was used for Western blotting.

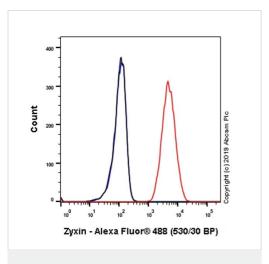
Blocking Buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM/TBST.

Observed band size: 82 kDa

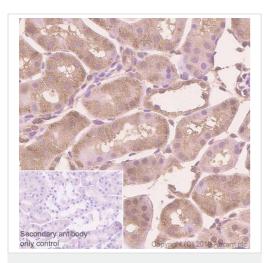
We are unsure about the extra bands. They might be the cleavage fragments as what are described in PMID:17572661

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab109316).



Flow Cytometry (Intracellular) - Anti-Zyxin antibody [EPR4302] - BSA and Azide free (ab238430)

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[®] 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). This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab109316**).

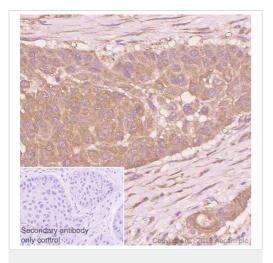


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Zyxin antibody

[EPR4302] - BSA and Azide free (ab238430)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of mouse kidney tissue sections labeling Zyxin with Purified <u>ab109316</u> 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 (<u>ab209101</u>) was used as the secondary antibody. Negative control: PBS instead of the primary antibody. Hematoxylin was used as a counterstain.

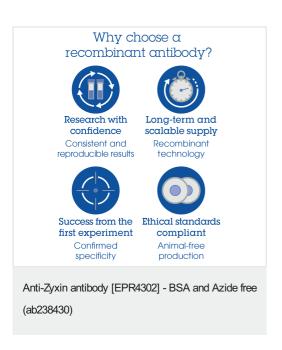
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab109316</u>).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Zyxin antibody
[EPR4302] - BSA and Azide free (ab238430)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human gastric carcinoma tissue sections labeling Zyxin with Purified <u>ab109316</u> 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 (<u>ab209101</u>) was used as the secondary antibody. Negative control: PBS instead of the primary antibody. Hematoxylin was used as a counterstain.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab109316).



Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

Our Abpromise to you: Quality guaranteed and expert technical support

- · Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit https://www.abcam.co.jp/abpromise or contact our technical team.

Terms and conditions

• Guarantee only valid for products bought direct from Abcam or one of our authorized distributors