abcam

Product datasheet

Anti-alpha Actinin 4 antibody [EPR2533(2)] ab108198



★★★★★ 9 Abreviews 26 References 画像数 11

製品の概要

製品名 Anti-alpha Actinin 4 antibody [EPR2533(2)]

製品の詳細 Rabbit monoclonal [EPR2533(2)] to alpha Actinin 4

由来種 Rabbit

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

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: Human skeletal muscle, A431, Mouse brain, Rat brain, Rat Heart, HAP1 and MFC7 Lysates

Flow Cyt: HeLa IHC-P: Human breast carcinoma, Mouse and Rat liver tissues ICC/IF: HAP1 cells

(HAP1-ACTN4 knockout cells used as negative cell line), MCF7 IP: HeLa cell lysate

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.

Stable for 12 months at -20°C.

バッファー pH: 7.20

Preservative: 0.01% Sodium azide

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

精製度 Protein A purified

ポリ/モノ モノクローナル クローン名 EPR2533(2)

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ab108198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
WB	****(4)	1/1000 - 1/10000. Detects a band of approximately 100 kDa (predicted molecular weight: 105 kDa).
IP		1/30.
IHC-P		1/100 - 1/250. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
ICC/IF	* * * * <u>(3)</u>	1/100 - 1/250.
Flow Cyt (Intra)		1/50. <u>ab172730</u> - Rabbit monoclonal lgG, is suitable for use as an isotype control with this antibody.

ターゲット情報

機能 F-actin cross-linking protein which is thought to anchor actin to a variety of intracellular structures.

This is a bundling protein. Probably involved in vesicular trafficking via its association with the CART complex. The CART complex is necessary for efficient transferrin receptor recycling but not

for EGFR degradation.

組織特異性 Widely expressed.

関連疾患 Defects in ACTN4 are the cause of focal segmental glomerulosclerosis type 1 (FSGS1)

[MIM:603278]. A renal pathology defined by the presence of segmental sclerosis in glomeruli and resulting in proteinuria, reduced glomerular filtration rate and edema. Renal insufficiency often progresses to end-stage renal disease, a highly morbid state requiring either dialysis therapy or

kidney transplantation.

配列類似性 Belongs to the alpha-actinin family.

Contains 1 actin-binding domain.

Contains 2 CH (calponin-homology) domains.

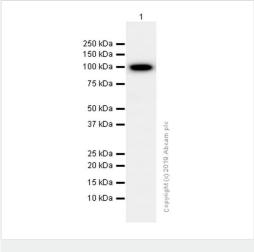
Contains 2 EF-hand domains.
Contains 4 spectrin repeats.

細胞内局在 Nucleus. Cytoplasm. Localized in cytoplasmic mRNP granules containing untranslated mRNAs.

Colocalizes with actin stress fibers. Nuclear translocation can be induced by the PI3 kinase inhibitor wortmannin or by cytochalasin D. Exclusively localized in the nucleus in a limited number

of cell lines.

画像



Western blot - Anti-alpha Actinin 4 antibody [EPR2533(2)] (ab108198)

All lanes : Anti-alpha Actinin 4 antibody [EPR2533(2)] (ab108198) at 1/1000 dilution (Purified)

Anti-alpha Actinin 4 antibody [EPR2533(2)] (ab108198) at 1/10000 dilution (Purified) + HeLa (Human cervix adenocarcinoma epithelial

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

Lane 1: Human skeletal muscle lysate

Lane 2: A431 (Human epidermoid carcinoma epithelial cell) whole cell lysate

Lane 3: Mouse brain lysate

cell) whole cell lysate at 15 µg

Predicted band size: 105 kDa

Observed band size: 105 kDa

Secondary

Lane 4: Rat brain lysate

Lane 5: Rat heart lysate

Lysates/proteins at 20 µg per lane.

1 2 3 4 5

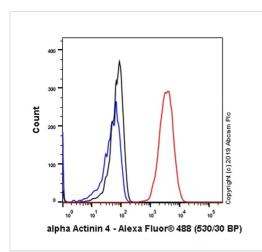
250 kDa —
150 kDa —
75 kDa —
37 kDa —
25 kDa —
20 kDa —
15 kDa —
10 kDa —
10 kDa —

Western blot - Anti-alpha Actinin 4 antibody [EPR2533(2)] (ab108198)

Secondary

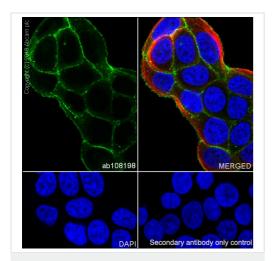
All lanes : Goat Anti-Rabbit IgG (HRP) with minimal cross-reactivity with human IgG at 1/2000 dilution

Predicted band size: 105 kDa **Observed band size:** 105 kDa



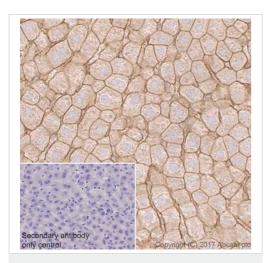
Flow Cytometry (Intracellular) - Anti-alpha Actinin 4 antibody [EPR2533(2)] (ab108198)

Flow Cytometry analysis of HeLa (Human cervix adenocarcinoma epithelial cell) cells labelling alpha Actinin 4 with Purified ab108198 at 1:50 dilution (10 μ g/ml) (Red). Cells were fixed with 4% Paraformaldehyde and permeabilised with 90% Methanol. A Goat anti rabbit lgG (Alexa Fluor® 488, <u>ab150077</u>) secondary antibody was used at 1:2000. Isotype control - Rabbit monoclonal lgG (Black). Unlabelled control - Cell without incubation with primary antibody and secondary antibody (Blue).



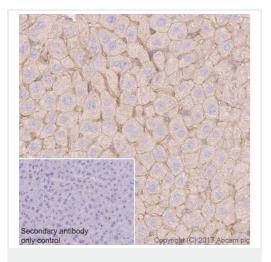
Immunocytochemistry/ Immunofluorescence - Antialpha Actinin 4 antibody [EPR2533(2)] (ab108198)

Immunocytochemistry analysis of MCF7 (Human breast adenocarcinoma epithelial cell) cells labeling alpha Actinin 4 with Purified ab108198 at 1:250 dilution (2.0 μ g/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 μ g/ml). Goat anti rabbit lgG (Alexa Fluor® 488, **ab150077**) was used as the secondary antibody at 1:1000 (2 μ g/ml) dilution. DAPI (blue) was used as nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.



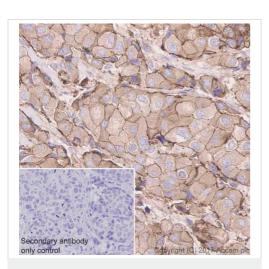
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-alpha Actinin 4 antibody [EPR2533(2)] (ab108198)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of rat liver tissue sections labeling alpha Actinin 4 with Purified ab108198 at 1:150 dilution (3.29 µg/mL). Heat mediated antigen retrieval was performed using Perform heat mediated antigen retrieval using **ab93684** (Tris/EDTA buffer, pH 9.0). Tissue was counterstained with Hematoxylin. ImmunoHistoProbe one step HRP Polymer (ready to use) secondary antibody was used. PBS instead of the primary antibody was used as the negative control.



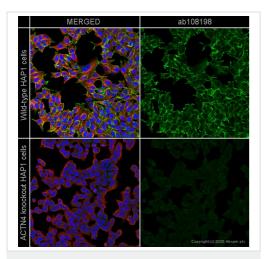
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-alpha Actinin 4 antibody [EPR2533(2)] (ab108198)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of mouse liver tissue sections labeling alpha Actinin 4 with Purified ab108198 at 1:150 dilution (3.29 µg/mL). Heat mediated antigen retrieval was performed using Perform heat mediated antigen retrieval using ab93684 (Tris/EDTA buffer, pH 9.0). Tissue was counterstained with Hematoxylin. ImmunoHistoProbe one step HRP Polymer (ready to use) secondary antibody was used as the negative control.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-alpha Actinin 4 antibody [EPR2533(2)] (ab108198)

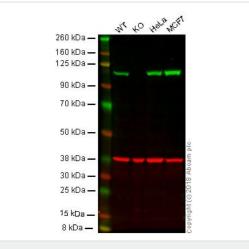
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human breast carcinoma tissue sections labeling alpha Actinin 4 with Purified ab108198 at 1:150 dilution (3.29 µg/mL). Heat mediated antigen retrieval was performed using Perform heat mediated antigen retrieval using **ab93684** (Tris/EDTA buffer, pH 9.0). Tissue was counterstained with Hematoxylin. ImmunoHistoProbe one step HRP Polymer (ready to use) secondary antibody was used. PBS instead of the primary antibody was used as the negative control.



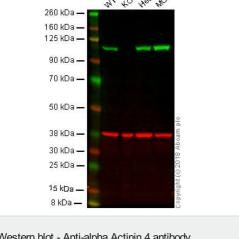
Immunocytochemistry/ Immunofluorescence - Antialpha Actinin 4 antibody [EPR2533(2)] (ab108198)

ab108198 staining ACTN4 in wild-type HAP1 cells (top panel) and ACTN4 knockout HAP1 cells (bottom panel). The cells were fixed with PFA (10min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with ab108198 at 1/100 dilution and ab7291 (Tubulin) at 1/1000 dilution overnight at +4°C, followed by a further incubation at room temperature for 1h with a goat secondary antibody to rabbit lgG (Alexa Fluor® 488) (ab150081) at 2 μ g/ml (shown in green) and a goat secondary antibody to mouse lgG (Alexa Fluor® 594) (ab150120) at 2 μ g/ml (shown in pseudo color red). Nuclear DNA was labelled in blue with DAPI.

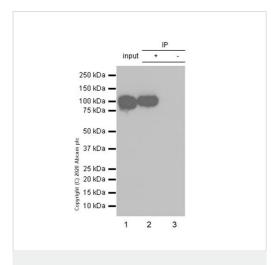
Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).



[EPR2533(2)] (ab108198)



Western blot - Anti-alpha Actinin 4 antibody



Immunoprecipitation - Anti-alpha Actinin 4 antibody [EPR2533(2)] (ab108198)

Lane 1: Wild-type HAP1 whole cell lysate (20 µg)

Lane 2: ACTN4 (alpha Actinin 4) knockout HAP1 whole cell lysate $(20 \mu g)$

Lane 3: HeLa whole cell lysate (20 µg)

Lane 4: MCF7 whole cell lysate (20 µg)

Lanes 1 - 4: Merged signal (red and green). Green - ab108198 observed at 105 kDa. Red - loading control, ab9484, observed at 37 kDa.

ab108198 was shown to specifically react with alpha Actinin 4 in wild-type HAP1 cells as signal was lost in ACTN4 (alpha Actinin 4) knockout cells. Wild-type and ACTN4 (alpha Actinin 4) knockout samples were subjected to SDS-PAGE. ab108198 and ab9484 (Mouse anti-GAPDH loading control) were incubated overnight at 4°C at 1/1000 dilution and 1/20000 dilution respectively. Blots were developed with Goat anti-Rabbit lgG H&L (IRDye® 800CW) preabsorbed ab216773 and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed ab216776 secondary antibodies at 1/20000 dilution for 1 hour at room temperature before imaging.

Purified ab108198 at 1/50 dilution (2µg) immunoprecipitating alpha Actinin 4 in HeLa whole cell lysate.

Lane 1 (input): HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate 10µg

Lane 2 (+): ab108198 + HeLa whole cell lysate.

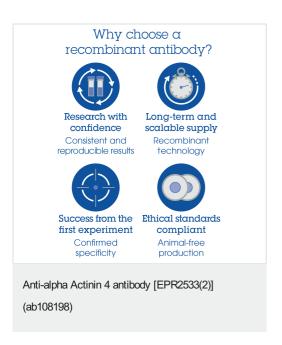
Lane 3 (-): Rabbit monoclonal IgG (ab172730) instead of ab108198 in HeLa whole cell lysate.

VeriBlot for IP Detection Reagent (HRP) (ab131366) (1/1000 dilution) was used for Western blotting.

Blocking Buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM/TBST.

Observed band size: 105 kDa



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