abcam

Product datasheet

Anti-Ezrin antibody [3C12] ab4069



★★★★★ 8 Abreviews 56 References

画像数 6

製品の概要

製品名 Anti-Ezrin antibody [3C12]

製品の詳細 Mouse monoclonal [3C12] to Ezrin

由来種 Mouse

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

種交差性 交差種: Mouse, Human

交差が予測される動物種: Rat, Hamster, Cow, Pig, Kangaroo, Monkey

免疫原 Recombinant fragment corresponding to Human Ezrin aa 350-600.

ポジティブ・コントロール Lung.

特記事項 This antibody clone is manufactured by Abcam. If you require a custom buffer formulation or

conjugation for your experiments, please contact orders@abcam.com.

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

製品の特性

製品の状態

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 Preservative: 0.02% Sodium azide

Constituents: PBS, 6.97% L-Arginine

精製度 Protein G purified

ポリ/モノ モノクローナル

クローン名 3C12

アイソタイプ

lgG1

軽鎖の種類

kappa

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ab4069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	★★★★ (5)	1/100 - 1/750. Predicted molecular weight: 69 kDa.
IHC-Fr	★★☆☆☆(1)	1/50 - 1/100.
Flow Cyt (Intra)		Use 1µg for 10 ⁶ cells. ab170190 - Mouse monoclonal lgG1, is suitable for use as an isotype control with this antibody.
IHC-P		Use a concentration of 0.025 - 1 µg/ml.

ターゲット情報

機能 Probably involved in connections of major cytoskeletal structures to the plasma membrane. In

epithelial cells, required for the formation of microvilli and membrane ruffles on the apical pole.

Along with PLEKHG6, required for normal macropinocytosis.

組織特異性 Expressed in cerebral cortex, basal ganglia, hippocampus, hypophysis, and optic nerve. Weakly

expressed in brain stem and diencephalon. Stronger expression was detected in gray matter of frontal lobe compared to white matter (at protein level). Component of the microvilli of intestinal epithelial cells. Preferentially expressed in astrocytes of hippocampus, frontal cortex, thalamus, parahippocampal cortex, amygdala, insula, and corpus callosum. Not detected in neurons in most

tissues studied.

配列類似性 Contains 1 FERM domain.

発生段階 Very strong staining is detected in the Purkinje cell layer and in part of the molecular layer of the

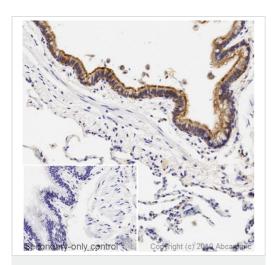
infant brain compared to adult brain.

翻訳後修飾 Phosphorylated by tyrosine-protein kinases.

細胞内局在 Apical cell membrane. Cell projection. Cell projection > microvillus membrane. Cell projection >

ruffle membrane. Cytoplasm > cell cortex. Cytoplasm > cytoskeleton. Localization to the apical membrane of parietal cells depends on the interaction with MPP5. Localizes to cell extensions and peripheral processes of astrocytes (By similarity). Microvillar peripheral membrane protein.

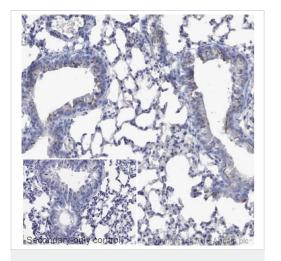
画像



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Ezrin antibody [3C12] (ab4069)

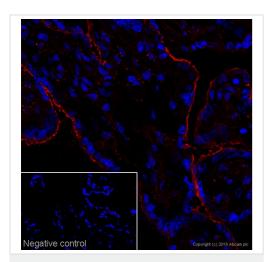
IHC image of Ezrin staining in a section of formalin-fixed paraffinembedded normal human lung performed on a Leica BONDTM system using the standard protocol F. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20mins. The section was then incubated with ab4069, 0.025ug/ml, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX. The inset secondary-only control image is taken from an identical assay without primary antibody.

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.

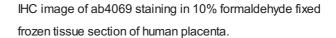


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Ezrin antibody [3C12] (ab4069)

IHC image of Ezrin staining in mouse lung formalin fixed paraffin embedded tissue section. The section was pre-treated using pressure cooker heat mediated antigen retrieval with sodium citrate buffer (pH6) for 30mins. The section was incubated with ab4069, 1µg/ml overnight at +4°C. An HRP-conjugated secondary (Ab97040, 1/1000 dilution) was used for 1hr at room temperature. The section was counterstained with haematoxylin and mounted with DPX.



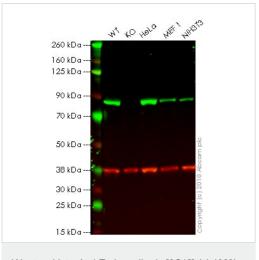
Immunohistochemistry (Frozen sections) - Anti-Ezrin antibody [3C12] (ab4069)



Non-specific protein-protein interactions were blocked using TBS containing 0.025% (v/v) Triton X-100, 0.3M (w/v) glycine and 3% (w/v) BSA for 1 hour at room temperature. The section was then incubated with ab4069 (1 μ g/ml dilution) in TBS containing 0.025% (v/v) Triton X-100 and 3% (w/v) BSA overnight at +4°C. The section was then incubated with <u>ab150119</u> (Goat polyclonal Secondary Antibody to Mouse IgG - H&L (Alexa Fluor[®] 647)) and DAPI for 1 hour at room temperature.

The DAPI only control (no antibody) inset shows no autofluorescence, demonstrating that any Alexa Fluor[®] 647 signal is derived directly from bound ab4069.

For other IHC staining systems (automated and non-automated), customers should optimize variable parameters such as antibody concentrations and incubation times.



Western blot - Anti-Ezrin antibody [3C12] (ab4069)

All lanes: Anti-Ezrin antibody [3C12] (ab4069) at 1 µg/ml

Lane 1: Wild-type HAP1 whole cell lysate

Lane 2: EZR (Ezrin) knockout HAP1 whole cell lysate

Lane 3 : HeLa whole cell lysate

Lane 4: MEF whole cell lysate

Lane 5: NIH3T3 whole cell lysate

Lysates/proteins at 20 µg per lane.

Predicted band size: 69 kDa

Lanes 1 - 5: Merged signal (red and green). Green - ab4069 observed at 81 kDa. Red - loading control, **ab181602**, observed at 37 kDa.

ab4069 was shown to specifically react with Ezrin in wild-type HAP1 cells as signal was lost in EZR (Ezrin) knockout cells. Wild-type and EZR (Ezrin) knockout samples were subjected to SDS-PAGE. Ab4069 and ab181602 (Rabbit anti-GAPDH loading control) were incubated overnight at 4°C at 1 µg/ml and 1/20000 dilution respectively. Blots were developed with Goat anti-Mouse IgG H&L (IRDye® 800CW) preabsorbed ab216772 and Goat anti-Rabbit IgG H&L (IRDye® 680RD) preabsorbed ab216777 secondary antibodies at 1/20000 dilution for 1 hour at room temperature before imaging.

Anti-Ezrin antibody [3C12] (ab4069) at 1/500 dilution + Mouse bEnd.3 whole cell lysate at 20 µg

Secondary

HRP-conjugated goat anti-mouse IgG polyclonal at 1/10000 dilution

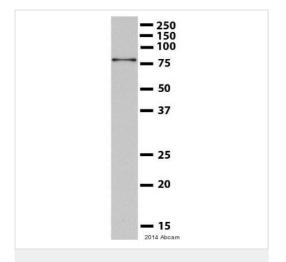
Developed using the ECL technique.

Performed under reducing conditions.

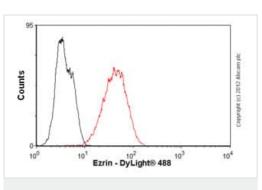
Predicted band size: 69 kDa **Observed band size:** 81 kDa

Exposure time: 10 seconds

Overlay histogram showing SH-SY5Y cells stained with ab4069 (red line). The cells were fixed with 4% paraformaldehyde (10 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab4069, $1\mu g/1x10^6$ cells) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-mouse IgG (H+L) (ab96879) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was mouse IgG1 [ICIGG1] (ab91353, $2\mu g/1x10^6$ cells) used under the same conditions. Acquisition of >5,000 events was performed. This antibody gave a positive signal in SH-SY5Y cells fixed with 80% methanol (5 min)/permeabilized with 0.1% PBS-Tween for 20 min used under the same conditions.



Western blot - Anti-Ezrin antibody [3C12] (ab4069)
This image is courtesy of an anonymous Abreview



Flow Cytometry (Intracellular) - Anti-Ezrin antibody [3C12] (ab4069)

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