abcam

ab4069

Anti-Ezrin antibody [3C12]

KO Validated

Key facts

Isotype	IgG1
Host species	Mouse
Storage buffer	Preservative: 0.02% Sodium azide Constituents: PBS, 6.97% L-Arginine
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Clone number	3C12
Purification technique	Affinity purification
Light chain type	kappa
Concentration	1 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	1 µg for 10^6 Cells

Notes	ab170190 - Mouse monoclonal IgG1, is suitable for use as an isotype control with this antibody.

Expected

Species	Mouse
Dilution info	Use at an assay dependent concentration.
Notes	-

Predicted

Species	Rat, Hamster, Cow, Pig, Kangaroo, Monkey
Dilution info	-
Notes	-

WB

Tested

Species	Human
Dilution info	1/100 - 1/750
Notes	-

Expected

Species	Mouse
Dilution info	Use at an assay dependent concentration.
Notes	-

Predicted

Species	Rat, Hamster, Cow, Pig, Kangaroo, Monkey
Dilution info	-
Notes	-

IHC-P

Tested

Species	Mouse
Dilution info	0.025-1 µg/mL
Notes	-

Species	Human
Dilution info	0.025-1 µg/mL
Notes	-

Predicted

Species	Rat, Hamster, Cow, Pig, Kangaroo, Monkey
Dilution info	-
Notes	-

IHC-Fr

Tested

Species	Human
Dilution info	1/50 - 1/100
Notes	-

Expected

Species	Mouse
Dilution info	Use at an assay dependent concentration.
Notes	-

Predicted

Species	Rat, Hamster, Cow, Pig, Kangaroo, Monkey
Dilution info	-
Notes	-

Target data

See full target information EZR

Function	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.

Storage

Shipped at conditions	Blue Ice
Appropriate short-term storage duration	1-2 weeks
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C
Aliquoting information	Upon delivery aliquot
Storage information	Avoid freeze / thaw cycle

Notes

Want a custom formulation?
This antibody clone is manufactured by Abcam. If you require a custom buffer formulation or conjugation for your experiments, please contact orders@abcam.com

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	Ezrin also known as cytovillin or villin-2 is a protein belonging to the ezrin-radixin-moesin (ERM) family. It is approximately 81 kDa in molecular weight. Ezrin plays a critical role in linking the actin cytoskeleton to the plasma membrane. This interaction occurs through its ability to bind to membrane proteins and phospholipids facilitating structural support and signal transduction. Ezrin is widely expressed in epithelial tissues but it also occurs in other cell types such as lymphocytes and osteoclasts.
Biological function summary	Ezrin functions as a membrane-microfilament linker and is essential for maintaining cell shape adhesion and motility. It is an integral component of many cellular structures including microvilli and ruffles. Ezrin's activity often involves

	forming complexes with other proteins such as EBP50 and F-actin which are fundamental for its role in cellular morphogenesis. These interactions allow ezrin to regulate membrane surface structure and cell surface organization.
Pathways	Ezrin participates in various signaling pathways that are essential for cell migration and invasion. One key pathway is the Rho family of GTPases which regulates actin cytoskeleton dynamics. Ezrin interacts with proteins like Rho-associated kinase (ROCK) that influence cell contraction and motility. Additionally ezrin is involved in the MAPK/ERK pathway which influences cell growth and survival by interacting with other signaling molecules.
Associated diseases and disorders	Ezrin shows significant relevance to cancer progression and metastasis particularly in conditions like osteosarcoma. Ezrin overexpression correlates with increased metastatic potential and poor clinical outcomes. It also has implications in allergic diseases due to its role in immune cell function. In these contexts ezrin interacts with SRC family kinases which are important for transmitting signals from cell-surface receptors to downstream pathways influencing disease development and progression.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

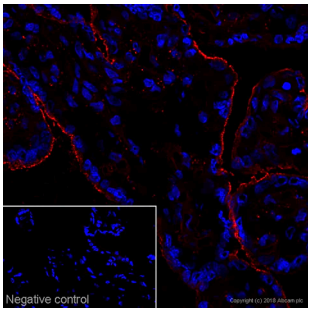
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

7 product images



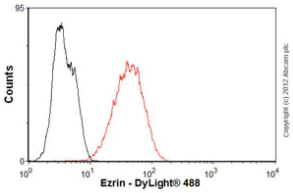
Immunohistochemistry (Frozen sections) - Anti-Ezrin antibody [3C12] (ab4069)

Ezrin Immunohistochemistry (Frozen sections) staining using mouse Anti-Ezrin antibody

IHC image of ab4069 staining in 10% formaldehyde fixed frozen tissue section of human placenta. 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 (1µg/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 ab150119 (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.

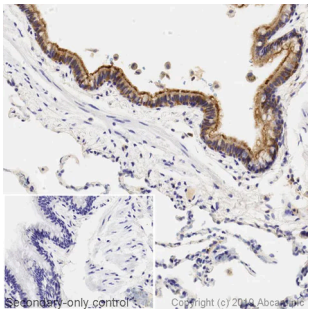
For other IHC staining systems (automated and non-automated), customers should optimize variable parameters such as antibody concentrations and incubation times.



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

Ezrin Flow Cytometry (Intracellular) staining using mouse Anti-Ezrin antibody

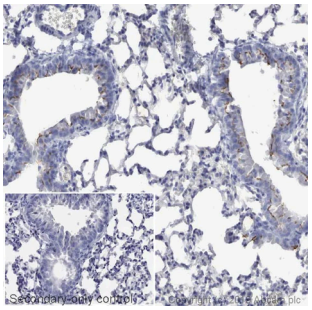
Overlay histogram showing SH-SY5Y cells stained with ab4069 (red line). The cells were fixed with 4% paraformaldehyde (10 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab4069, 1µg/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, 2µg/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.



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

Ezrin Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using mouse Anti-Ezrin antibody

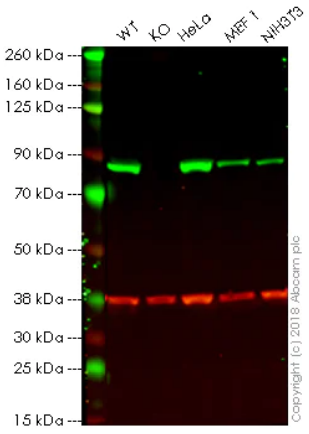
IHC image of Ezrin staining in a section of formalin-fixed paraffin-embedded normal human lung performed on a Leica BOND™ system using the standard protocol F. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20mins. 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 paraffin-embedded sections) - Anti-Ezrin antibody [3C12] (ab4069)

Ezrin Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using mouse Anti-Ezrin antibody

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.



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

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.
All lanes:
Western blot - Anti-Ezrin antibody [3C12] (ab4069) at 1 µg/mL

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

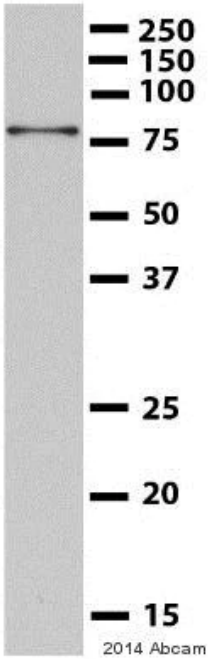
Lane 2:
EZR (Ezrin) knockout HAP1 whole cell lysate at 20 µg

Lane 3:
HeLa whole cell lysate at 20 µg

Lane 4:
MEF whole cell lysate at 20 µg

Lane 5:
NIH3T3 whole cell lysate at 20 µg

Predicted band size: 69 kDa



This image is courtesy of an anonymous customer review

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

All lanes:
Western blot - Anti-Ezrin antibody [3C12] (ab4069) at 1/500 dilution

All lanes:
Mouse bEnd.3 whole cell lysate at 20 µg

Secondary

All lanes:
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: 10s

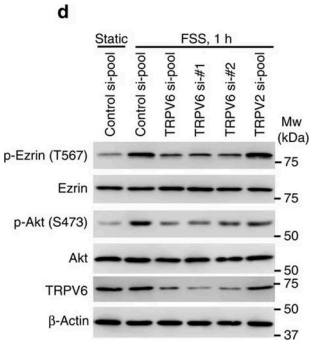


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Western blot - Anti-Ezrin antibody [3C12] (ab4069)

Ezrin Western blot staining using mouse Anti-Ezrin antibody

Ezrin western blot using anti-Ezrin antibody [3C12] ab4069. Publication image and figure legend from Miura, S., Sato, K., et al., 2015, Nat Commun, PubMed 26563429.

ab4069 was used in this publication in western blot. This may not be the same as the application(s) guaranteed by Abcam. For a full list of applications guaranteed by Abcam for ab4069 please see the product overview.

Essential roles of TRPV6 in FSS-induced microvilli formation.(a) Apical localization of ezrin in the FSS-exposed BeWo cells. Cells were cultured overnight under static conditions, and then cultured with or without medium perfusion (5 µl min−1) for 1 h. Ezrin (green) localization was observed by immunofluorescence confocal microscopy. Nuclei were counterstained with 4′,6-diamidino-2-phenylindole (DAPI; blue). Serial x–y focal planes (z-sections, 0.88 µm interval in ‘Static', 0.90 µm in ‘Flow') and the stacked images are shown. Scale bar, 20 µm. (b) Time-course phosphorylation of Ezrin (pThr567) and Akt (pSer473) in response to FSS. BeWo cells were cultured overnight under static conditions and exposed to FSS (5 µl min−1) for the indicated times. The cells were lysed, and protein expression level or phosphorylation was analysed by immunoblot. (c) Inhibition of Akt phosphorylation by BAPTA-AM. Gö6793 (100 nM), BAPTA-AM (10 µM) or buffer control (DMSO) was added to the perfusing medium. (d) Involvement of TRPV6 in the FSS-induced Ezrin phosphorylation. BeWo cells transfected with siRNA were seeded in the chamber area of the device and cultured overnight under static conditions before exposure to FSS for 1 h. β-Actin was used as a loading control. Note that the TRPV6 siRNA oligo (TRPV6 si-#1 or #2), as well as the siRNA pool, shows similar inhibitory effects on the FSS-induced phosphorylation of Ezrin and Akt. (e) Impaired Ezrin localization in the TRPV6 knockdown cells. Ezrin localization in the TRPV6 knockdown cells was analysed by immunofluorescence confocal microscopy. Note that TRPV6 knockdown cells exposed to FSS (5 µl min−1) for 1 h failed to show the microvillous localization pattern of Ezrin as observed in the control siRNA cells under FSS. Scale bar, 20 µm.

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