

Product datasheet

Anti-Ezrin antibody [EP886Y] - Plasma Membrane Marker ab40839

KO VALIDATED Recombinant RabMAB

★★★★★ [5 Abreviews](#) [16 References](#) [17 Images](#)

Overview

Product name	Anti-Ezrin antibody [EP886Y] - Plasma Membrane Marker
Description	Rabbit monoclonal [EP886Y] to Ezrin - Plasma Membrane Marker
Host species	Rabbit
Tested applications	Suitable for: ICC/IF, Flow Cyt (Intra), WB, IP, IHC-P
Species reactivity	Reacts with: Human
Immunogen	Synthetic peptide within Human Ezrin aa 450-550 (C terminal). The exact sequence is proprietary.
Positive control	WB: Wild-type HAP1 whole cell lysate; HeLa and HCT116 whole cell lysates. IHC-P: Human Colon, breast and bladder carcinoma. ICC/IF: HeLa cells Flow Cyt (intra): HeLa cells IP: HeLa cells
General notes	<p>This product is a recombinant monoclonal antibody, which offers several advantages including:</p> <ul style="list-style-type: none">- High batch-to-batch consistency and reproducibility- Improved sensitivity and specificity- Long-term security of supply- Animal-free production <p>For more information see here.</p> <p>Our RabMAB[®] technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAB[®] patents.</p> <p>We are constantly working hard to ensure we provide our customers with best in class antibodies. As a result of this work we are pleased to now offer this antibody in purified format. We are in the process of updating our datasheets. The purified format is designated 'PUR' on our product labels. If you have any questions regarding this update, please contact our Scientific Support team.</p> <p>Mouse, Rat: We have preliminary internal testing data to indicate this antibody may not react with these species. Please contact us for more information.</p>

Properties

Form Liquid

Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol, 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EP886Y
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab40839 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
ICC/IF	★★★★★ (2)	1/500. For upurified use at 1/50 - 1/100
Flow Cyt (Intra)		1/800. ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody. For upurified use at 1/1000
WB	★★★★★ (2)	1/5000 - 1/50000. Detects a band of approximately 72 kDa (predicted molecular weight: 69 kDa).
IP		1/40. For upurified use at 1/50
IHC-P	★★★★★ (1)	1/250. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. See <u>IHC antigen retrieval protocols</u> .

Target

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.
Tissue specificity	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.
Sequence similarities	Contains 1 FERM domain.
Developmental stage	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.

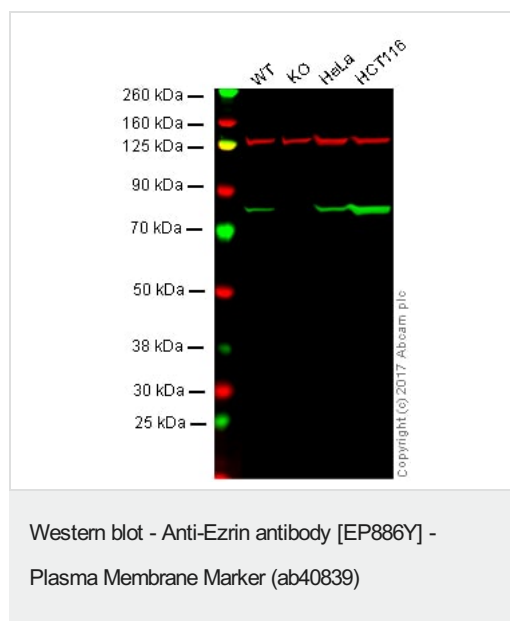
Post-translational modifications

Phosphorylated by tyrosine-protein kinases.

Cellular localization

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.

Images



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

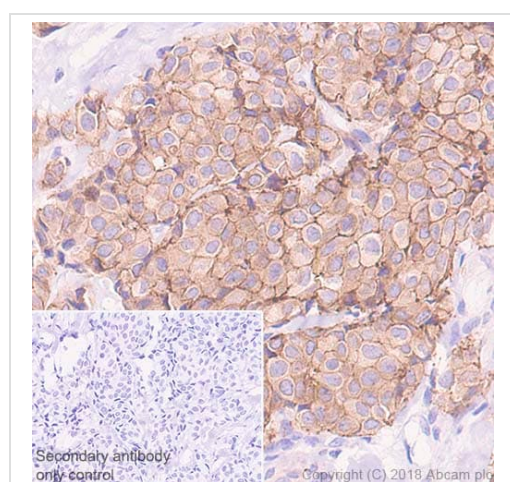
Lane 2: Ezrin knockout HAP1 whole cell lysate (20 µg)

Lane 3: HeLa whole cell lysate (20 µg)

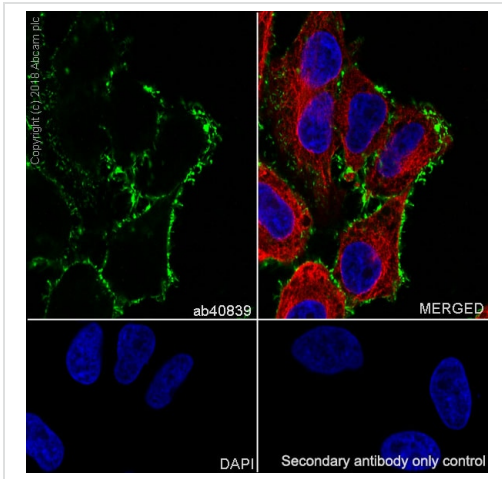
Lane 4: HCT116 whole cell lysate (20 µg)

Lanes 1 - 4: Merged signal (red and green). Green - ab40839 observed at 75 kDa. Red - loading control, **ab18058**, observed at 130 kDa.

ab40839 was shown to specifically react with Ezrin in wild-type HAP1 cells as signal was lost in Ezrin knockout cells. Wild-type and Ezrin knockout samples were subjected to SDS-PAGE. Ab40839 and **ab18058** (Mouse anti-Vinculin loading control) were incubated overnight at 4°C at 1/5000 dilution and 1/20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed **ab216773** and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed **ab216776** secondary antibodies at 1/10000 dilution for 1 hour at room temperature before imaging.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Human breast carcinoma tissue sections labeling Ezrin with Purified ab40839 at 1:250 dilution (3.28 µg/ml). Heat mediated antigen retrieval was performed Perform heat mediated antigen retrieval using **ab93684** (Tris/EDTA buffer, pH 9.0). ImmunoHistoProbe one step HRP Polymer (ready to use) was used as the secondary antibody. Negative control: PBS instead of the primary antibody. Hematoxylin was used as a counterstain.



Immunocytochemistry/ Immunofluorescence - Anti-Ezrin antibody [EP886Y] - Plasma Membrane Marker (ab40839)

Immunocytochemistry/ Immunofluorescence analysis of HeLa (Human cervix adenocarcinoma epithelial cell) cells labeling Ezrin with Purified ab40839 at 1:500 dilution (1.6 µg/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 µg/ml). Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) was used as the secondary antibody at 1:1000 (2 µg/ml) dilution. DAPI nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.



Western blot - Anti-Ezrin antibody [EP886Y] - Plasma Membrane Marker (ab40839)

All lanes : Anti-Ezrin antibody [EP886Y] - Plasma Membrane Marker (ab40839) at 1/20000 dilution (Purified)

Lane 1 : HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysates

Lane 2 : HCT 116 (Human colorectal carcinoma epithelial cell) whole cell lysates

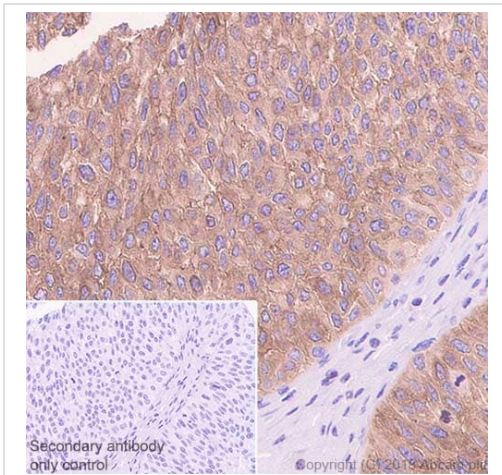
Lysates/proteins at 20 µg per lane.

Secondary

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

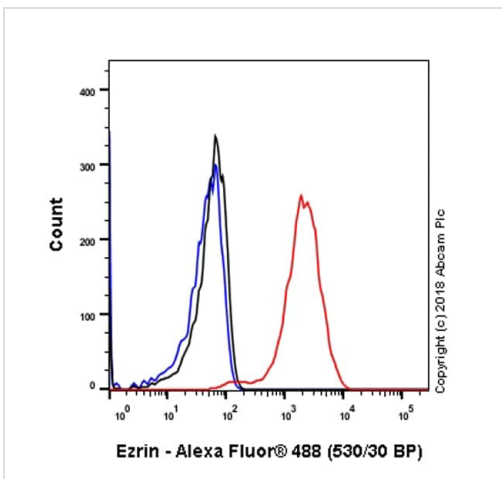
Predicted band size: 69 kDa

Observed band size: 72 kDa



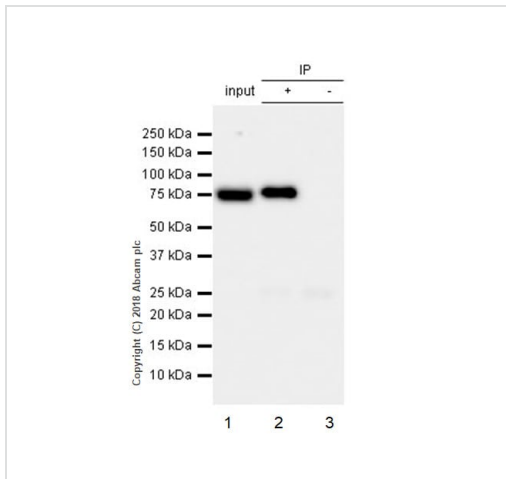
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Human bladder carcinoma tissue sections labeling Ezrin with Purified ab40839 at 1:250 dilution (3.28 µg/ml). Heat mediated antigen retrieval was performed Perform heat mediated antigen retrieval using **ab93684** (Tris/EDTA buffer, pH 9.0). ImmunoHistoProbe one step HRP Polymer (ready to use) was used as the secondary antibody. Negative control: PBS instead of the primary antibody. Hematoxylin was used as a counterstain.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Ezrin antibody [EP886Y] - Plasma Membrane Marker (ab40839)



Intracellular Flow Cytometry analysis of HeLa (Human cervix adenocarcinoma epithelial cell) cells labeling Ezrin with Purified ab40839 at 1/800 dilution (1 µg/ml) (red). Cells were fixed with 4% Paraformaldehyde. A Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) secondary antibody was used at 1/2000. Isotype control - Rabbit monoclonal IgG (Black). Unlabeled control - Cell without incubation with primary antibody and secondary antibody (Blue).

Flow Cytometry (Intracellular) - Anti-Ezrin antibody [EP886Y] - Plasma Membrane Marker (ab40839)



Immunoprecipitation - Anti-Ezrin antibody [EP886Y]
- Plasma Membrane Marker (ab40839)

ab40839 (purified) at 1:40 dilution (2µg) immunoprecipitating Ezrin in HeLa whole cell lysate.

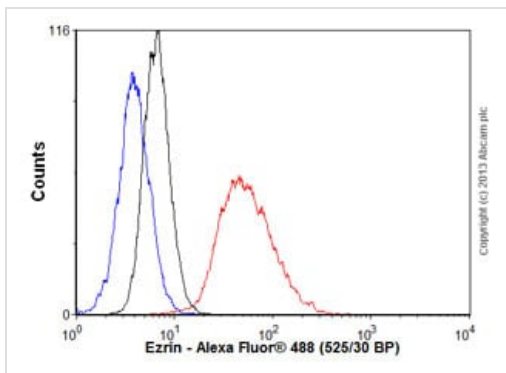
Lane 1 (input): HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate 10µg

Lane 2 (+): ab40839 & HeLa whole cell lysate

Lane 3 (-): Rabbit monoclonal IgG (**ab172730**) instead of ab40839 in HeLa whole cell lysate

For western blotting, VeriBlot for IP Detection Reagent (HRP) (**ab131366**) was used for detection at 1:1000 dilution.

Blocking and diluting buffer: 5% NFD/MTBST.



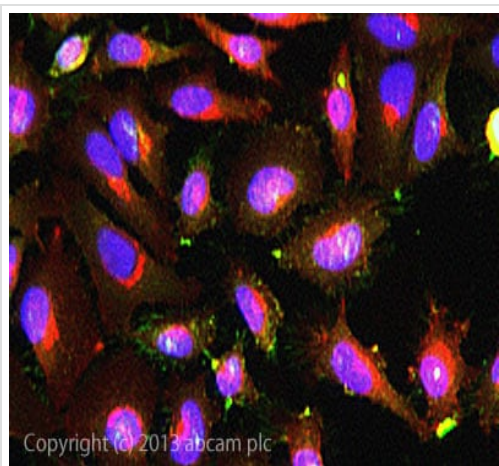
Flow Cytometry (Intracellular) - Anti-Ezrin antibody [EP886Y] - Plasma Membrane Marker (ab40839)

Overlay histogram showing SH-SY5Y cells stained with upurified ab40839 (red line). The cells were fixed with 80% methanol (5 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 (ab40839, 1/1000 dilution) for 30 min at 22°C. The secondary antibody used was Alexa Fluor® 488 goat anti-rabbit IgG (H&L) (**ab150077**) at 1/2000 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) (0.1µg/1x10⁶ cells) used under the same conditions.

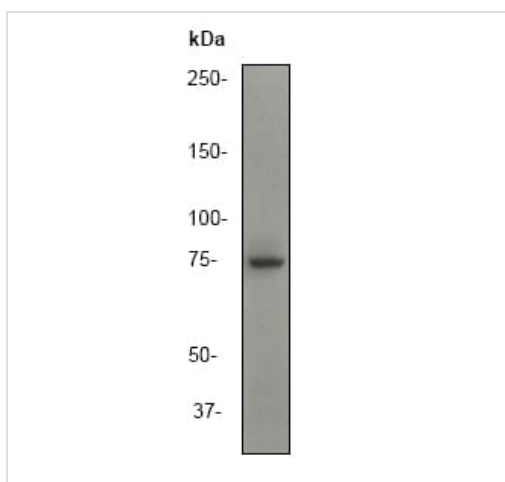
Unlabelled sample (blue line) was also used as a control.

Acquisition of >5,000 events were collected using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter. This antibody gave a positive signal in SH-SY5Y cells fixed with 4% paraformaldehyde (10 min)/permeabilized with 0.1% PBS-Tween for 20 min used under the same conditions.



Immunocytochemistry/ Immunofluorescence - Anti-Ezrin antibody [EP886Y] - Plasma Membrane Marker (ab40839)

ICC/IF image of unpurified ab40839 stained HeLa cells. The cells were 100% methanol fixed (5 min) and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody ab40839 at 1/100 dilution overnight at +4°C. The secondary antibody (green) was DyLight® 488 goat anti- rabbit ([ab96899](#)) IgG (H+L) used at a 1/250 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (red) at a 1/200 dilution for 1h. DAPI was used to stain the cell nuclei (blue) at a concentration of 1.43µM.

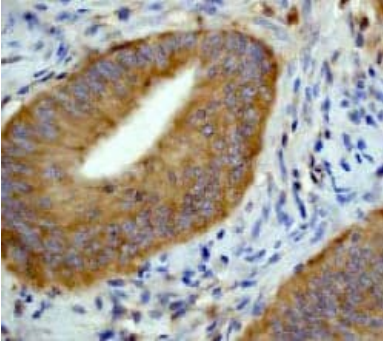


Western blot - Anti-Ezrin antibody [EP886Y] - Plasma Membrane Marker (ab40839)

Anti-Ezrin antibody [EP886Y] - Plasma Membrane Marker (ab40839) at 1/50000 dilution (Unpurified) + HeLa cell lysate at 10 µg

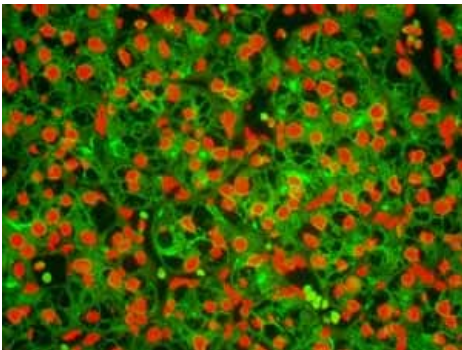
Predicted band size: 69 kDa

Observed band size: 72 kDa



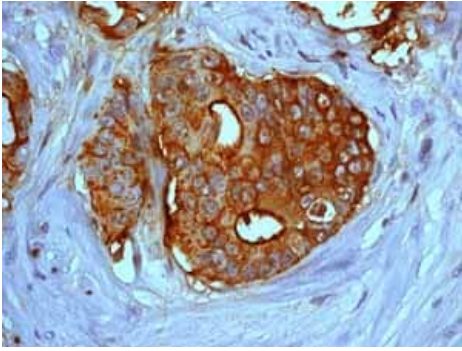
Unpurified ab40839 at a 1:100 dilution staining Ezrin in human colon carcinoma tissue.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Ezrin antibody [EP886Y]
- Plasma Membrane Marker (ab40839)



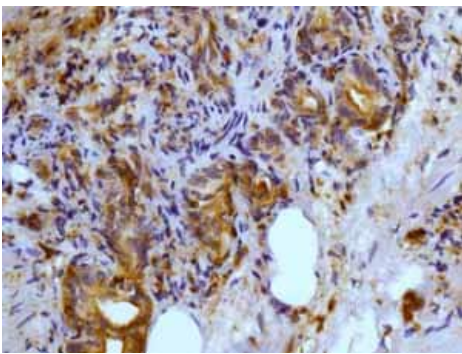
Fluorescent immunohistochemical analysis of paraffin-embedded human kidney carcinoma tissue using unpurified ab40839. Green-Ezrin red-PI

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Ezrin antibody [EP886Y]
- Plasma Membrane Marker (ab40839)



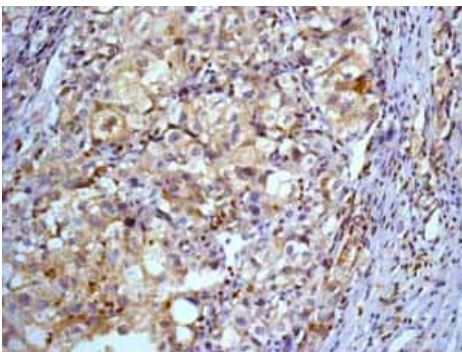
Unpurified ab40839 showing positive staining in Breast carcinoma tissue.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Ezrin antibody [EP886Y]
- Plasma Membrane Marker (ab40839)



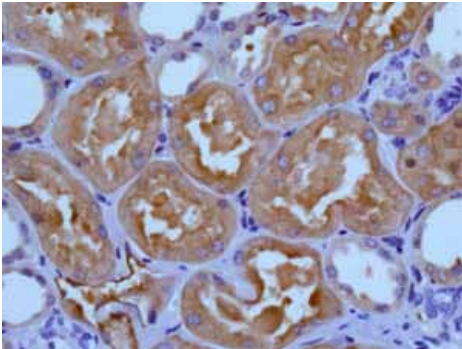
Unpurified ab40839 showing positive staining in Prostatic carcinoma tissue.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Ezrin antibody [EP886Y]
- Plasma Membrane Marker (ab40839)



Unpurified ab40839 showing positive staining in Hepatocellular carcinoma tissue.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Ezrin antibody [EP886Y]
- Plasma Membrane Marker (ab40839)



Unpurified ab40839 showing positive staining in Normal kidney tissue.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Ezrin antibody [EP886Y]
- Plasma Membrane Marker (ab40839)

Why choose a recombinant antibody?



Research with confidence
Consistent and reproducible results



Long-term and scalable supply
Recombinant technology



Success from the first experiment
Confirmed specificity



Ethical standards compliant
Animal-free production

Anti-Ezrin antibody [EP886Y] - Plasma Membrane Marker (ab40839)

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

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