abcam

Product datasheet

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



リコンピナント

RabMAb

★★★★★ 5 Abreviews 16 References 画像数 17

製品の概要

製品名 Anti-Ezrin antibody [EP886Y] - Plasma Membrane Marker

製品の詳細 Rabbit monoclonal [EP886Y] to Ezrin - Plasma Membrane Marker

由来種 Rabbit

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

種交差性 交差種: Human

免疫原 Synthetic peptide within Human Ezrin aa 450-550 (C terminal). The exact sequence is proprietary.

ポジティブ・コントロール 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

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**.

Mouse, Rat: We have preliminary internal testing data to indicate this antibody may not react with

these species. Please contact us for more information.

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.

Avoid freeze / thaw cycle.

バッファー pH: 7.20

Preservative: 0.01% Sodium azide

Constituents: PBS, 40% Glycerol, 0.05% BSA

1

精製度 Protein A purified

ポリ/モノ モノクローナル

クローン名 EP886Y

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ab40839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
ICC/IF	**** <u>(2)</u>	1/500. For upurified use at 1/50 - 1/100
Flow Cyt (Intra)		1/800. ab172730 - Rabbit monoclonal lgG, 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 IHC antigen retrieval protocols .

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.

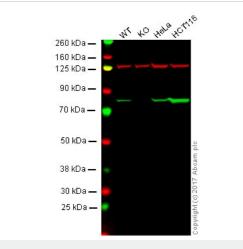
発生段階 Very strong staining is detected in the Purkinje cell layer and in part of the molecular layer of the

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.



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

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.

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

Lane 3: HeLa whole cell lysate (20 µg)

130 kDa.

before imaging.

Lane 4: HCT116 whole cell lysate (20 µg)

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

Lanes 1 - 4: Merged signal (red and green). Green - ab40839

ab40839 was shown to specifically react with Ezrin in wild-type

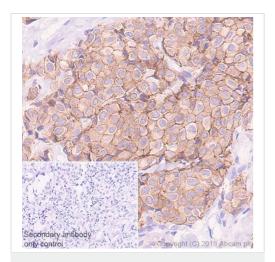
HAP1 cells as signal was lost in Ezrin knockout cells. Wild-type and

Ezrin knockout samples were subjected to SDS-PAGE. Ab40839 and ab18058 (Mouse anti-Vinculin loading control) were incubated

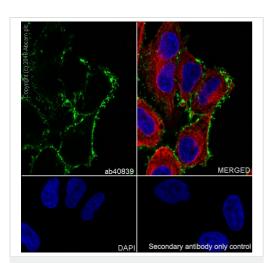
respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed ab216773 and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed ab216776 secondary antibodies at 1/10000 dilution for 1 hour at room temperature

overnight at 4°C at 1/5000 dilution and 1/20000 dilution

observed at 75 kDa. Red - loading control, ab18058, observed at

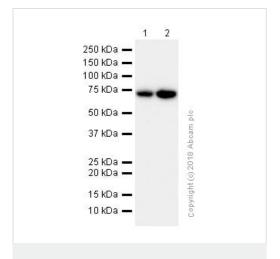


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Ezrin antibody [EP886Y] - Plasma Membrane Marker (ab40839)



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 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 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

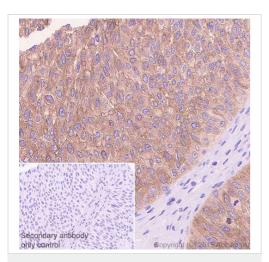
Lane 2: HCT 116 (Human colorectal carcinoma epithelial cell) whole cell lysates

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

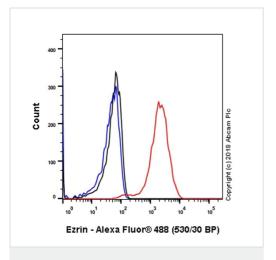
Predicted band size: 69 kDa **Observed band size:** 72 kDa



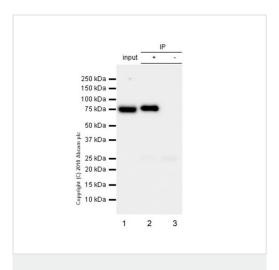
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Ezrin antibody [EP886Y]

- Plasma Membrane Marker (ab40839)

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.



Flow Cytometry (Intracellular) - 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 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).



Immunoprecipitation - Anti-Ezrin antibody [EP886Y]

- Plasma Membrane Marker (ab40839)



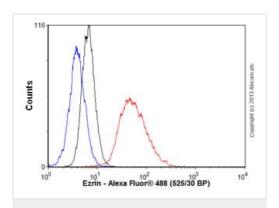
Lane 1 (input): HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate $10\mu g$

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

Lane 3 (-): Rabbit monoclonal lgG (<u>ab172730</u>) instead of ab40839 in HeLa whole cell lysate

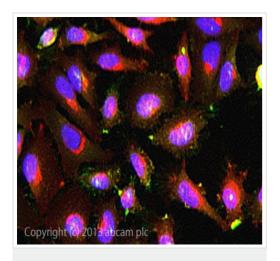
For western blotting, VeriBlot for IP Detection Reagent (HRP) (ab131366) was used for detection at 1:1000 dilution.

Blocking and diluting buffer: 5% NFDM/TBST.



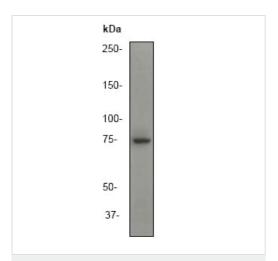
Flow Cytometry (Intracellular) - Anti-Ezrin antibody [EP886Y] - Plasma Membrane Marker (ab40839)

Overlay histogram showing SH-SY5Y cells stained with upurifiedab40839 (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)

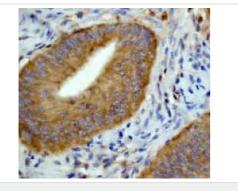
ICC/IF image of unpurified ab40839 stained HeLa cells. The cells were 100% methanol fixed (5 min) and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody ab40839 at 1/100 dilution overnight at +4°C. The secondary antibody (green) was DyLight® 488 goat anti- rabbit (ab96899) lgG (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 1.43 μ M.



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

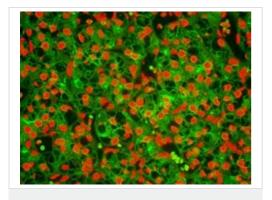
Predicted band size: 69 kDa **Observed band size:** 72 kDa



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Ezrin antibody [EP886Y]

- Plasma Membrane Marker (ab40839)

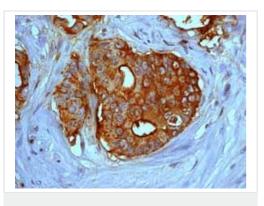
Unpurified ab40839 at a 1:100 dilution staining Ezrin in human colon carcinoma tissue.



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

Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Ezrin antibody [EP886Y]

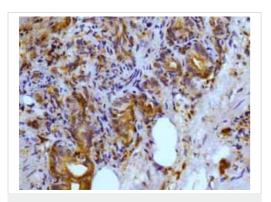
- Plasma Membrane Marker (ab40839)



Unpurified ab40839 showing positive staining in Breast carcinoma tissue.

Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Ezrin antibody [EP886Y]

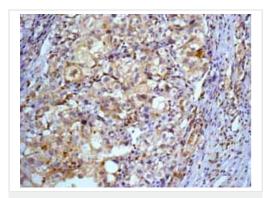
- Plasma Membrane Marker (ab40839)



Unpurified ab40839 showing positive staining in Prostatic carcinoma tissue.

Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Ezrin antibody [EP886Y]

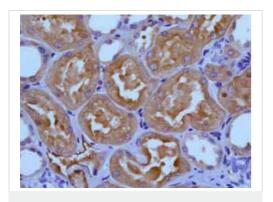
- Plasma Membrane Marker (ab40839)



Unpurified ab40839 showing positive staining in Hepatocellular carcinoma tissue.

Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Ezrin antibody [EP886Y]

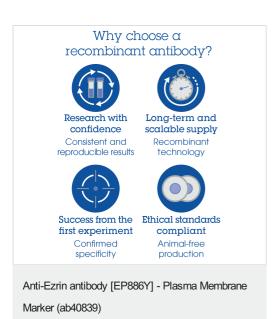
- Plasma Membrane Marker (ab40839)



Unpurified ab40839 showing positive staining in Normal kidney tissue.

Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Ezrin antibody [EP886Y]

- Plasma Membrane Marker (ab40839)



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