

Ezrin Polyclonal Antibody

Catalog NumberPA5-29358

Product data sheet

Details		Species Reactivity	
Size	100 µL	Species reactivity	Human, Mouse, Rat
Host/Isotope	Rabbit / IgG	Published species	Human
Class	Polyclonal	Tested Applications	
Type	Antibody	Immunohistochemistry (Paraffin) (IHC (P))	1:100-1:1,000
Immunogen	Recombinant fragment corresponding to a region within amino acids 103 and 444 of Human Ezrin	Western Blot (WB)	1:5,000-1:20,000
		Immunocytochemistry (ICC/IF)	1:100-1:1,000
Conjugate	Unconjugated	Published Applications	
Form	Liquid	Immunohistochemistry (IHC)	See 1 publications below
Concentration	1 mg/mL	* Suggested working dilutions are given as a guide only. It is recommended that the user titrate the product for use in their own experiment using appropriate negative and positive controls.	
Purification	Antigen affinity chromatography		
Storage buffer	PBS, pH 7, with 1% BSA, 20% glycerol		
Contains	0.01% thimerosal		
Storage Conditions	Store at 4°C short term. For long term storage, store at -20°C, avoiding freeze/thaw cycles.		

Product specific information

Recommended positive controls: 293T, A431, HeLa. Predicted reactivity: Mouse (96%), Rat (95%), Xenopus laevis (85%), Dog (98%), Rabbit (94%), Chicken (92%), Chimpanzee (99%), Bovine (96%). Store product as a concentrated solution. Centrifuge briefly prior to opening the vial.

Background/Target Information

The cytoplasmic peripheral membrane protein encoded by this gene functions as a protein-tyrosine kinase substrate in microvilli. As a member of the ERM protein family, this protein serves as an intermediate between the plasma membrane and the actin cytoskeleton. This protein plays a key role in cell surface structure adhesion, migration and organization, and it has been implicated in various human cancers. A pseudogene located on chromosome 3 has been identified for this gene. Alternatively spliced variants have also been described for this gene.

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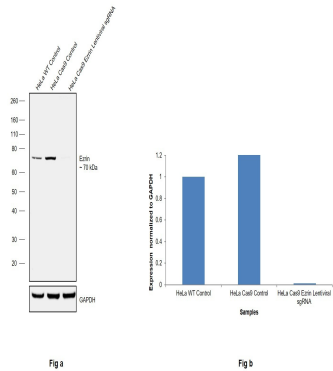
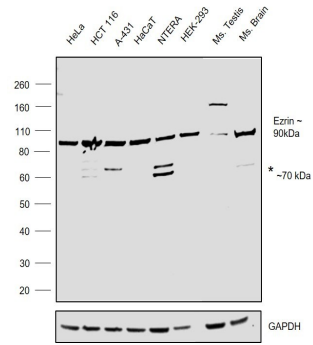
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Ezrin Antibody (PA5-29358) in WB

Western blot was performed using Anti-Ezrin Polyclonal Antibody (Product # PA5-29358) and a ~90kDa band corresponding to Ezrin was observed across cell lines and tissues tested . Membrane enriched extracts (30 µg lysate) of HeLa (Lane 1), HCT 116 (Lane 2), A-431 (Lane 3), HaCaT (Lane 4), NTERA-2 cl.D1 (Lane 5), HEK-293 (Lane 6), Mouse Testis (Lane 7), Mouse Brain (Lane 8) were electrophoresed using NuPAGE™ 4-12% Bis-Tris Protein Gel (Product # NP0321BOX). Resolved proteins were then transferred onto a Nitrocellulose membrane (Product # LC2001) by iBlot® 2 Dry Blotting System (Product # IB21001). The blot was probed with the primary antibody (1: 10000 dilution) and detected by chemiluminescence with Goat anti-Rabbit IgG (Heavy Chain) Superclonal™ Recombinant Secondary Antibody, HRP (Product # A27036, 1:4000) using the iBright FL 1000 (Product # A32752). Chemiluminescent detection was performed using Novex® ECL Chemiluminescent Substrate Reagent Kit (Product # WP20005). Another isoform around 70kDa was observed in some cell lines.

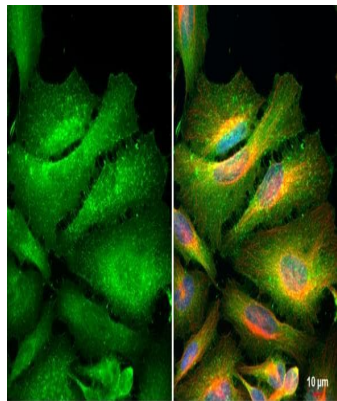
Ezrin Antibody (PA5-29358)

Antibody specificity was demonstrated by CRISPR-Cas9 mediated knockout of target protein. A loss of signal was observed for target protein in HeLa Cas9 cell line transduced with Ezrin Lentiviral sgRNA compared to control cell line using Anti-Ezrin Polyclonal Antibody (Product # PA5-29358). {KO}



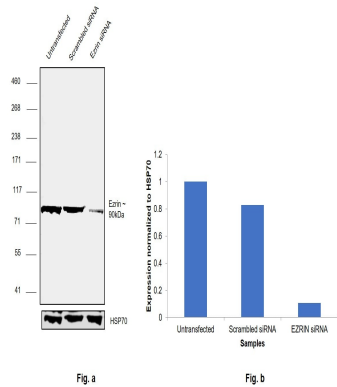
Ezrin Antibody (PA5-29358) in ICC/IF

Ezrin Polyclonal Antibody detects Ezrin protein at cell membrane by immunofluorescent analysis. Sample: HeLa cells were fixed in 4% paraformaldehyde at RT for 15 min. Green: Ezrin stained by Ezrin Polyclonal Antibody (Product # PA5-29358) diluted at 1:500. Red: alpha Tubulin, a cytoskeleton marker, stained by alpha Tubulin Polyclonal Antibody [GT114] (Product # MA5-31466) diluted at 1:1,000. Blue: Fluoroshield with DAPI .



Ezrin Antibody (PA5-29358)

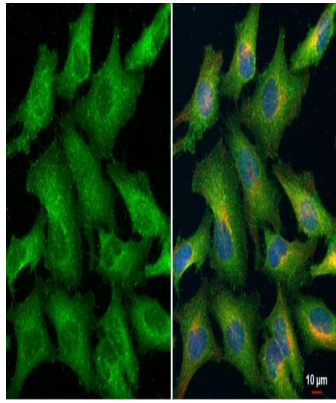
Antibody specificity was demonstrated by siRNA mediated knockdown of target protein. HCT 116 cells were transfected with Ezrin siRNA and decrease in signal intensity was observed in Western Blot application using Anti-Ezrin Polyclonal Antibody (Product # PA5-29358). {KD}



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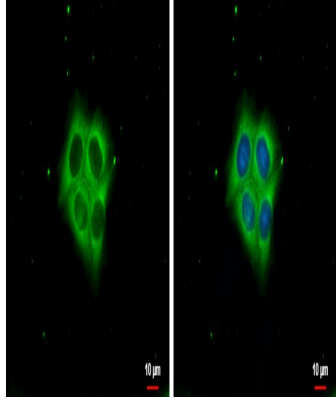
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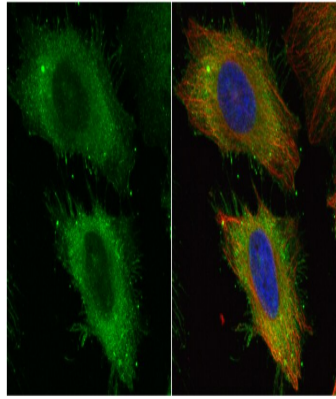
Ezrin Antibody (PA5-29358) in ICC/IF

Ezrin Polyclonal Antibody detects Ezrin protein at cell membrane by immunofluorescent analysis. Sample: HeLa cells were fixed in 4% paraformaldehyde at RT for 15 min. Green: Ezrin stained by Ezrin Polyclonal Antibody (Product # PA5-29358) diluted at 1:500. Red: alpha Tubulin, a cytoskeleton marker, stained by alpha Tubulin Polyclonal Antibody [GT114] (Product # MA5-31466) diluted at 1:1,000. Blue: Fluoroshield with DAPI .



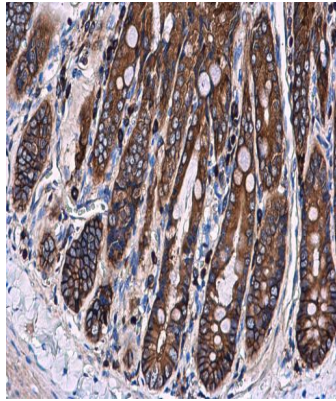
Ezrin Antibody (PA5-29358) in ICC/IF

Ezrin Polyclonal Antibody detects Ezrin protein in cytoplasm by immunofluorescent analysis. Sample: HCT116 cells were fixed in ice-cold MeOH for 5 min. Green: Ezrin protein stained by Ezrin Polyclonal Antibody (Product # PA5-29358) diluted at 1:1,000. Blue: Hoechst 33342 staining.



Ezrin Antibody (PA5-29358) in ICC/IF

Immunocytochemistry-Immunofluorescence analysis of Ezrin was performed in HeLa cells fixed in 4% paraformaldehyde at RT for 15 min. Green: Ezrin Polyclonal Antibody (Product # PA5-29358) diluted at 1:1000. Red: alpha Tubulin, a cytoskeleton marker. Blue: Hoechst 33342 staining.



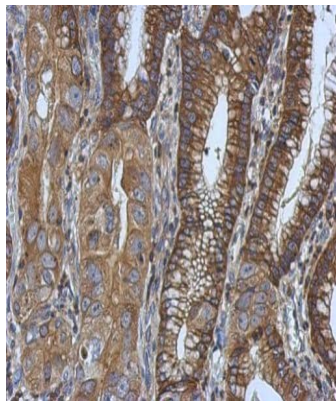
Ezrin Antibody (PA5-29358) in IHC (P)

Ezrin Polyclonal Antibody detects Ezrin protein at cytoplasm and membrane in rat duodenum by immunohistochemical analysis. Sample: Paraffin-embedded rat duodenum. Ezrin Polyclonal Antibody (Product # PA5-29358) diluted at 1:500. Antigen Retrieval: Citrate buffer, pH 6.0, 15 min.

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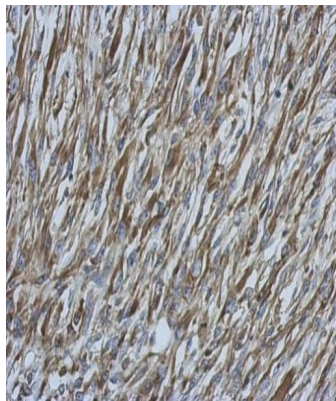
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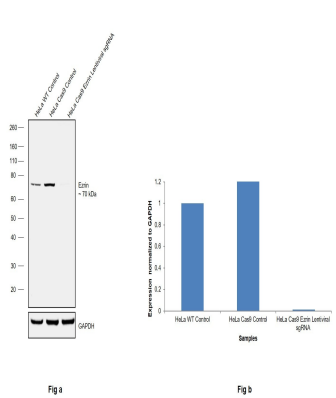
Ezrin Antibody (PA5-29358) in IHC (P)

Immunohistochemical analysis of paraffin-embedded human colon carcinoma, using Ezrin (Product # PA5-29358) antibody at 1:500 dilution. Antigen Retrieval: EDTA based buffer, pH 8.0, 15 min.



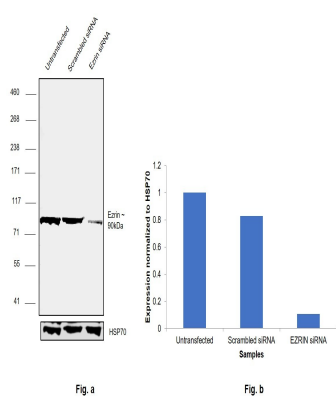
Ezrin Antibody (PA5-29358) in IHC (P)

Immunohistochemical analysis of paraffin-embedded C2C12 xenograft, using Ezrin (Product # PA5-29358) antibody at 1:500 dilution. Antigen Retrieval: EDTA based buffer, pH 8.0, 15 min.



Ezrin Antibody (PA5-29358) in WB

CRISPR-Cas9 mediated genome editing of Ezrin (as confirmed by next generation sequencing) was achieved by using LentiArray™ Lentiviral sgRNA (Product # A32042, AssayID CRISPR864181_LV) and LentiArray Cas9 Lentivirus (Product # A32064). Fig (a) Western blot analysis of Ezrin was performed by loading 30 µg of HeLa Wild Type (Lane 1), HeLa Cas9 (Lane 2) and HeLa Cas9 cells transduced with Ezrin Lentiviral sgRNA (Lane 3) whole cell extracts. The samples were electrophoresed using NuPAGE™ Novex™ 4-12% Bis-Tris Protein Gel (Product # NP0322BOX). Resolved proteins were then transferred onto a nitrocellulose membrane (Product # IB23001) by iBlot® 2 Dry Blotting System (Product # IB21001). The blot was probed with Anti-Ezrin Polyclonal Antibody (Product # PA5-29358) using 1:10,000 dilution and Goat anti-Rabbit IgG (Heavy Chain) Superclonal™ Recombinant Secondary Antibody, HRP (Product # A27036 1:8,000 dilution). Chemiluminescent detection was performed using Novex® ECL Chemiluminescent Substrate Reagent Kit (Product # WP20005). Loss of signal in sgRNA transduced cells using the LentiArray™ CRISPR product line confirms that antibody is specific to Ezrin (Fig (b)).



Ezrin Antibody (PA5-29358) in WB

Knockdown of Ezrin was achieved by transfecting HCT 116 with Ezrin specific siRNAs (Silencer® select Product # s14796, s14795). Western blot analysis (Fig. a) was performed using Membrane enriched extracts from the Ezrin knockdown cells (lane 3), non-targeting scrambled siRNA transfected cells (lane 2) and untransfected cells (lane 1). The blot was probed with Ezrin Polyclonal Antibody (Product # PA5-29358, 1:10000) and Goat anti-Rabbit IgG (Heavy Chain) Superclonal™ Recombinant Secondary Antibody, HRP (Product # A27036, 1:4000). Densitometric analysis of this western blot is shown in histogram (Fig. b). Decrease in signal upon siRNA mediated knock down confirms that antibody is specific to Ezrin.

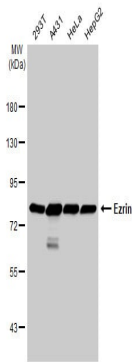
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Ezrin Antibody (PA5-29358) in WB

Western Blot using Ezrin Polyclonal Antibody (Product # PA5-29358). Various whole cell extracts (30 µg) were separated by 7.5% SDS-PAGE, and the membrane was blotted with Ezrin Polyclonal Antibody (Product # PA5-29358) diluted at 1:10,000. The HRP-conjugated anti-rabbit IgG antibody was used to detect the primary antibody.



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PubMed References For Ezrin Polyclonal Antibody

1 Immunohistochemistry References

Species / Dilution	Summary
	PA5-29358 was used in Immunohistochemistry to show that intravital 3D bioprinting of donor-muscle-derived stem cells under the epimysium of hindlimb muscle in mice leads to the de novo formation of myofibres in the mice.
Human / 1:100	<p>Nature biomedical engineering (2020; 4: 901)</p> <p>"Intravital three-dimensional bioprinting."</p> <p>Author(s):Urciuolo A,Poli I,Brandolino L,Raffa P,Scattolini V,Laterza C,Giobbe GG,Zambaiti E,Selmin G,Magnussen M,Brigo L,De Coppi P,Salmaso S,Giomo M,Elvassore N</p> <p>PubMed Article URL:http://dx.doi.org/10.1038/s41551-020-0568-z</p>

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