abcam

Product datasheet

Anti-PODXL antibody [EPR9518] ab150358

KO VALIDATED Recombinant RabMAb

3 Abreviews 12 References 18 Images

Overview

Product name	Anti-PODXL antibody [EPR9518]
Description	Rabbit monoclonal [EPR9518] to PODXL
Host species	Rabbit
Tested applications	**Suitable for:** Flow Cyt (Intra), WB, IHC-P, ICC/IF **Unsuitable for:** IP
Species reactivity	**Reacts with:** Human
Immunogen	Recombinant fragment within Human PODXL aa 300-500. The exact sequence is proprietary. Database link: **O00592**
Positive control	WB: Raji, HeLa, HCT116 and HAP1 whole cell lysate. Human fetal kidney lysate. IHC-P: Human kidney tissue. Human hepatocellular carcinoma, breast carcinoma and endometrial carcinoma tissue. Human glioma tissue. ICC/IF: HeLa cells. Flow Cyt (intra): HeLa cells.
General notes	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.

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	Preservative: 0.01% Sodium azide Constituents: 0.05% BSA, 40% Glycerol, PBS
Purity	Protein A purified

Clonality	Monoclonal
Clone number	EPR9518
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab150358 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
Flow Cyt (Intra)		1/250.
WB		1/1000 - 1/10000. Detects a band of approximately 165 kDa (predicted molecular weight: 58 kDa).
IHC-P	★★★★★ (2)	1/1000. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. **For unpurified use at 1/250 - 1/500.**
ICC/IF	★★★★★ (1)	1/100. **For unpurified use at 1/500.**

Application notes Is unsuitable for IP.

Target

Function Involved in the regulation of both adhesion and cell morphology and cancer progression. Function as an anti-adhesive molecule that maintains an open filtration pathway between neighboring foot processes in the podocyte by charge repulsion. Acts as a pro-adhesive molecule, enhancing the adherence of cells to immobilized ligands, increasing the rate of migration and cell-cell contacts in an integrin-dependent manner. Induces the formation of apical actin-dependent microvilli. Involved in the formation of a preapical plasma membrane subdomain to set up inital epithelial polarization and the apical lumen formation during renal tubulogenesis. Plays a role in cancer development and aggressiveness by inducing cell migration and invasion through its interaction with the actin-binding protein EZR. Affects EZR-dependent signaling events, leading to increased activities of the MAPK and PI3K pathways in cancer cells.

Tissue specificity Glomerular epithelium cell (podocyte).

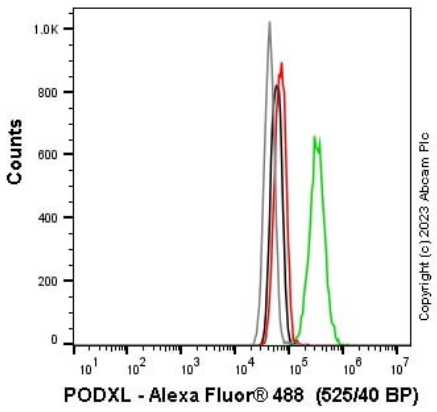
Sequence similarities Belongs to the podocalyxin family.

Domain Both the O-glycan-rich domain of the extracellular domain and the C-terminus PDZ-binding motif (DTHL) in the cytoplasmic tail harbor an apical sorting signal. The cytoplasmic domain is necessary for the apical membrane targeting and renal tubulogenesis. The cytoplasmic C-terminus PDZ-binding motif (DTHL) is essential for interaction with SLC9A3R1 and for targeting SLC9A3R1 to the apical cell membrane. The extracellular domain is necessary for microvillus formation (By similarity). The large highly anionic extracellular domain allows to maintain open filtration pathways between neighboring podocyte foot processes.

Post-translational modifications N- and O-linked glycosylated. Sialoglycoprotein.

Cellular localization	Apical cell membrane. Cell projection, lamellipodium. Cell projection, filopodium. Cell projection, ruffle. Cell projection, microvillus. Membrane raft. Membrane. In single attached epithelial cells is restricted to a preapical pole on the free plasma membrane whereas other apical and basolateral proteins are not yet polarized. Colocalizes with SLC9A3R2 at the apical plasma membrane during epithelial polarization. Colocalizes with SLC9A3R1 at the trans-Golgi network (transiently) and at the apical plasma membrane. Its association with the membrane raft is transient. Colocalizes with actin filaments, EZR and SLC9A3R1 in a punctate pattern at the apical cell surface where microvilli form. Colocalizes with EZR and SLC9A3R2 at the apical cell membrane of glomerular epithelium cells (By similarity). Forms granular, punctuated pattern, forming patches, preferentially adopting a polar distribution, located on the migrating poles of the cell or forming clusters along the terminal ends of filipodia establishing contact with the endothelial cells. Colocalizes with the submembrane actin of lamellipodia, particularly associated with ruffles. Colocalizes with vinculin at protrusions of cells. Colocalizes with ITGB1. Colocalizes with PARD3, PRKCI, EXOC5, OCLN, RAB11A and RAB8A in apical membrane initiation sites (AMIS) during the generation of apical surface and luminogenesis (By similarity).
Form	There are 2 isoforms produced by alternative splicing.

Images



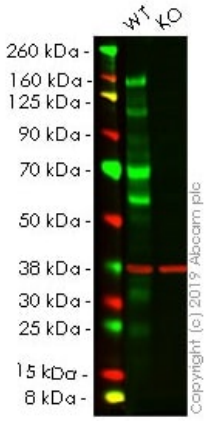
Flow Cytometry (Intracellular) - Anti-PODXL antibody [EPR9518] (ab150358)

Flow cytometry overlay histogram showing wild-type Hela (green line) and PODXL knockout Hela stained with ab150358 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilised with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS containing 10% normal goat serum to block non-specific protein-protein interaction followed by the antibody (ab150358) (1x 10^6 in 100µl at 1.0 µg/ml (1/2250)) for 30min at 22°C.

The secondary antibody Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed was incubated at 1/4000 for 30min at 22°C

Isotype control antibody Recombinant Rabbit IgG, monoclonal [EPR25A] - Isotype Control was used at the same concentration and conditions as the primary antibody (wild-type Hela - black line, PODXL knockout Hela - grey line). Unlabelled sample was also used as a control (this line is not shown for the purpose of simplicity).

Acquisition of >5000 events were collected using a 50 mW Blue laser (488nm) and 525/40 bandpass filter.



Western blot - Anti-PODXL antibody [EPR9518] (ab150358)

All lanes : Anti-PODXL antibody [EPR9518] (ab150358) at 1/10000 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : PODXL knockout HeLa cell lysate

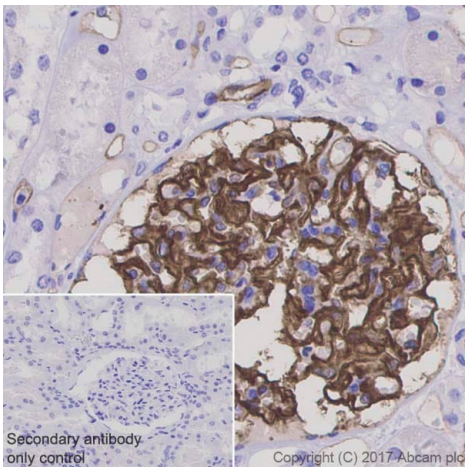
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 58 kDa
Observed band size: 160 kDa

Lanes 1- 2: Merged signal (red and green). Green - ab150358 observed at 160 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) observed at 37 kDa.

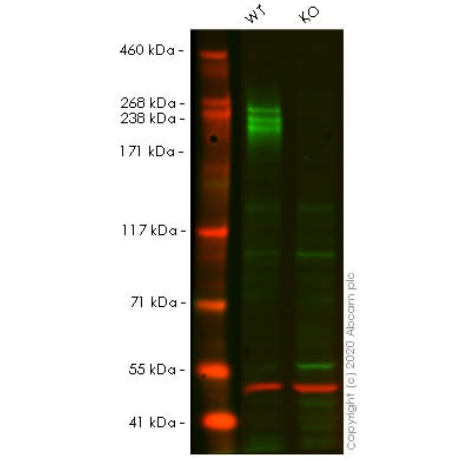
ab150358 was shown to react with PODXL in wild-type HeLa cells in western blot. Loss of signal was observed when knockout cell line **ab264984** (knockout cell lysate **ab257210**) was used. Wild-type HeLa and PODXL knockout HeLa cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. ab150358 and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) overnight at 4°C at a 1 in 10000 dilution and a 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye®800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye®680RD) preadsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PODXL antibody [EPR9518] (ab150358)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human kidney tissue sections labeling PODXL with purified ab150358 at 1/1000 dilution (0.44 µg/ml). Heat mediated antigen retrieval was performed using **ab93684** (Tris/EDTA buffer, pH 9.0). Tissue was counterstained with hematoxylin. ImmunoHistoProbe one step HRP Polymer (ready to use) is the secondary antibody.

PBS instead of the primary antibody was used as the negative control.



Western blot - Anti-PODXL antibody [EPR9518] (ab150358)

All lanes : Anti-PODXL antibody [EPR9518] (ab150358) at 1/1000 dilution

Lane 1 : Wild-type HCT116 cell lysate
Lane 2 : PODXL knockout HCT116 cell lysate

Lysates/proteins at 20 µg per lane.

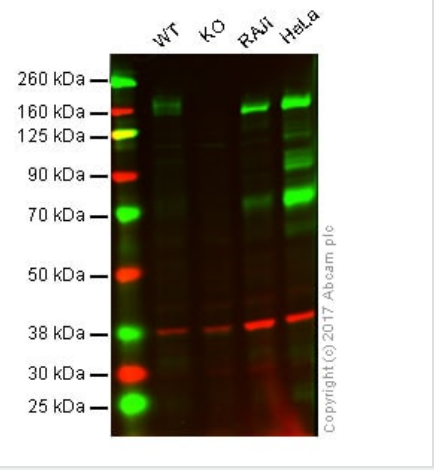
Performed under reducing conditions.

Predicted band size: 58 kDa
Observed band size: 200 kDa

Lanes 1- 2: Merged signal (red and green). Green - ab150358 observed at 200 kDa. Red - Anti-alpha Tubulin antibody [DM1A] - Loading Control (**ab7291**) observed at 50 kDa.

ab150358 was shown to react with PODXL in wild-type HCT116 cells in western blot. Loss of signal was observed when knockout cell line **ab266887** (knockout cell lysate **ab257211**) was used. Wild-Type HCT116 and PODXL knockout HCT116 cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at

room temperature in 0.1% TBST with 3% non-fat dried milk. ab150358 and Anti-alpha Tubulin antibody [DM1A] - Loading Control (**ab7291**) overnight at 4°C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye®800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye®680RD) preadsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Western blot - Anti-PODXL antibody [EPR9518] (ab150358)

All lanes : Anti-PODXL antibody [EPR9518] (ab150358) at 1/1000 dilution

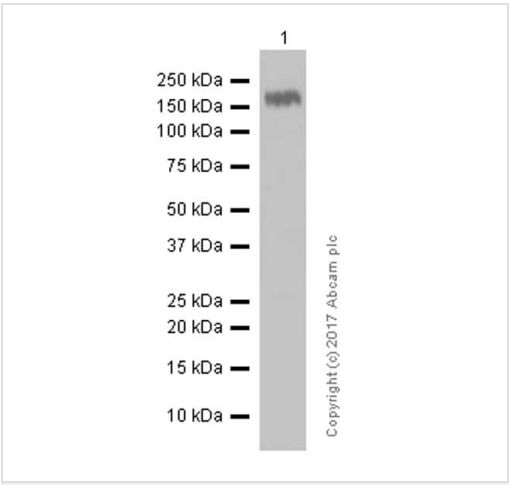
Lane 1 : Wild-type HAP1 whole cell lysate
Lane 2 : PODXL knockout HAP1 whole cell lysate
Lane 3 : Raji whole cell lysate
Lane 4 : HeLa whole cell lysate

Lysates/proteins at 20 µg per lane.

Predicted band size: 58 kDa

Lanes 1 - 4: Merged signal (red and green). Green - ab150358 observed at 160 kDa. Red - loading control, **ab9484**, observed at 37 kDa.

ab150358 was shown to specifically react with PODXL in wild-type cells as signal was lost in PODXL knockout cells. Wild-type and PODXL knockout samples were subjected to SDS-PAGE. Ab150358 and **ab9484** (Mouse anti-GAPDH loading control) were incubated overnight at 4°C at a 1/1000 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 1/20000 dilution for 1 hour at room temperature before imaging.



Western blot - Anti-PODXL antibody [EPR9518] (ab150358)

Anti-PODXL antibody [EPR9518] (ab150358) at 1/10000 dilution (purified) + Human fetal kidney lysates at 15 µg

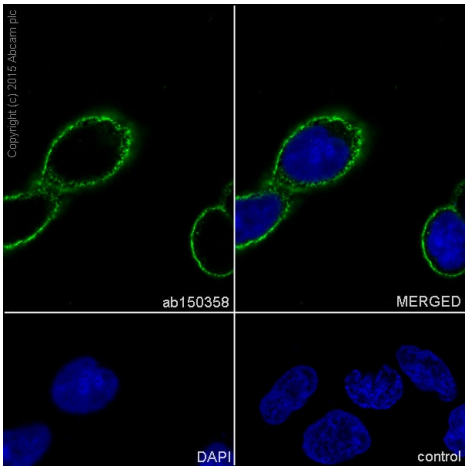
Secondary

Anti-Rabbit IgG (HRP), specific to the non-reduced form of IgG at 1/2000 dilution

Predicted band size: 58 kDa

Observed band size: 165 kDa

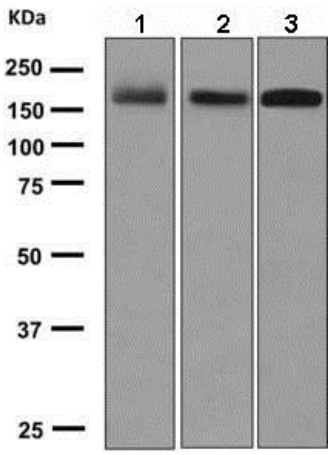
Blocking and diluting buffer: 5% NFDM/TBST.



Immunocytochemistry/ Immunofluorescence - Anti-PODXL antibody [EPR9518] (ab150358)

Immunocytochemistry/Immunofluorescence analysis of HeLa (human cervix adenocarcinoma) cells labeling PODXL with purified ab150358 at 1/500. Cells were fixed with 100% methanol. An Alexa Fluor® 488-conjugated goat anti-rabbit IgG (**ab150077**) at 1/1000 dilution was used as the secondary antibody. Nuclei couterstained with DAPI (blue).

Secondary Only Control: PBS was used instead of the primary antibody as the negative control.



Western blot - Anti-PODXL antibody [EPR9518] (ab150358)

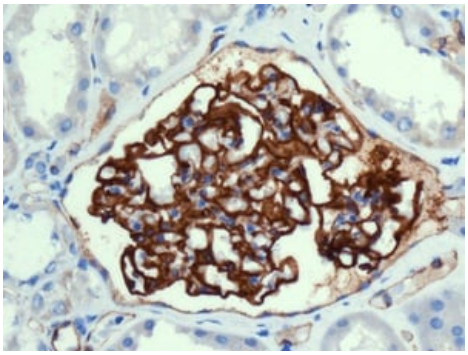
All lanes : Anti-PODXL antibody [EPR9518] (ab150358) at 1/1000 dilution (unpurified)

Lane 1 : Raji lysate
Lane 2 : HeLa lysate
Lane 3 : Human fetal kidney lysate

Lysates/proteins at 10 µg per lane.

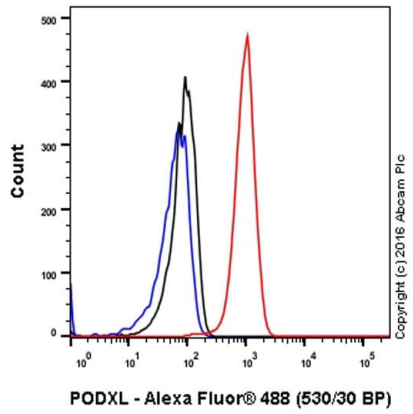
Secondary
All lanes : HRP labelled goat anti-rabbit at 1/2000 dilution

Predicted band size: 58 kDa
Observed band size: 165 kDa



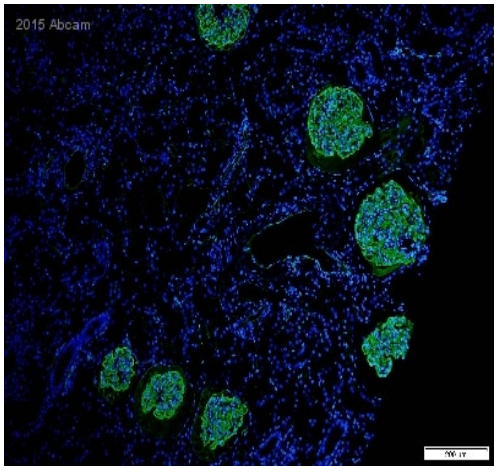
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PODXL antibody [EPR9518] (ab150358)

Immunohistochemical analysis of paraffin embedded human kidney tissue labeling PODXL with unpurified ab150358 antibody at a dilution of 1/100. Heat mediated antigen retrieval was performed before commencing with IHC staining protocol.



Flow Cytometry (Intracellular) - Anti-PODXL antibody [EPR9518] (ab150358)

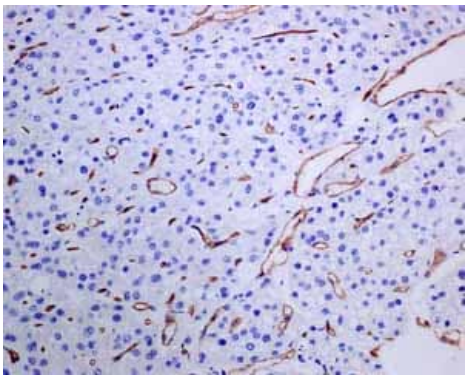
Intracellular Flow Cytometry analysis of HeLa (human cervix adenocarcinoma) cells labeling PODXL with purified ab150358 at 1/250 dilution (red). The secondary antibody was Goat anti rabbit IgG (Alexa Fluor® 488) at 1/2000 dilution. A Rabbit monoclonal IgG (Black) was used as the isotype control and cells without incubation with primary antibody and secondary antibody (Blue) were used as unlabeled control.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PODXL antibody [EPR9518] (ab150358)

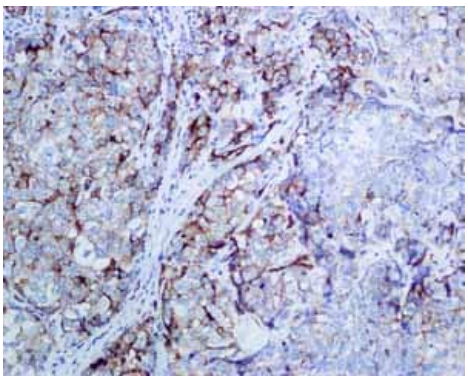
This image is courtesy of an anonymous abreview.

Unpurified ab150358 staining PODXL in human kidney tissue sections by Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections). Tissue samples were fixed with formaldehyde, cut into 20 micron slices, permeabilized with 0.05% tween-20 and blocked for 60 minutes at 25°C. Antigen retrieval was by heat mediation. The sample was incubated with primary antibody at a dilution of 1/250 at 25°C for 1 hour. An Alexa Fluor® 488-conjugated donkey anti-rabbit polyclonal (1/1000) was used as the secondary antibody, at a dilution of 1/1200.



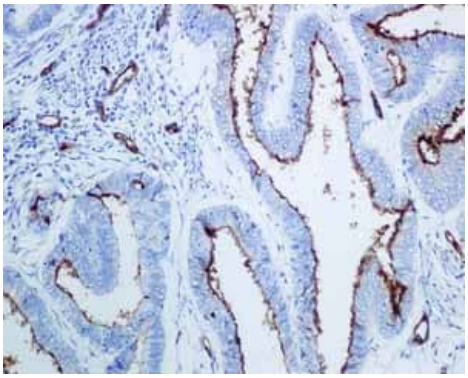
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PODXL antibody [EPR9518] (ab150358)

Immunohistochemical analysis of paraffin embedded human hepatocellular carcinoma vessels using unpurified ab150358 showing positive staining. Heat mediated antigen retrieval was performed before commencing with IHC staining protocol.



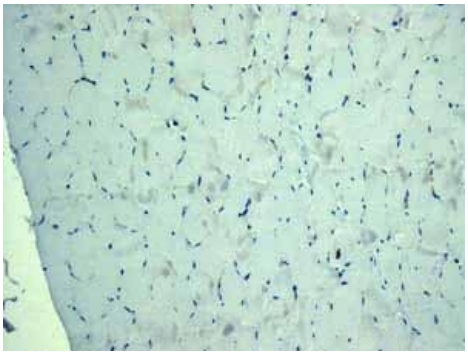
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PODXL antibody [EPR9518] (ab150358)

Immunohistochemical analysis of paraffin embedded human breast adenocarcinoma tissue using unpurified ab150358 showing positive staining. Heat mediated antigen retrieval was performed before commencing with IHC staining protocol.



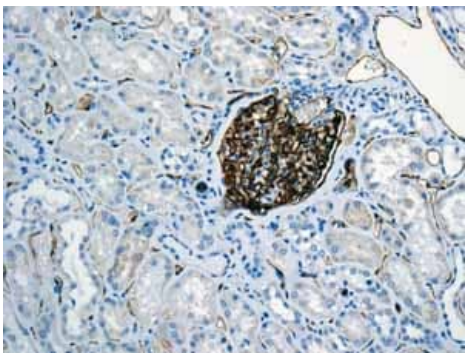
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PODXL antibody [EPR9518] (ab150358)

Immunohistochemical analysis of paraffin embedded human endometrial carcinoma tissue using unpurified ab150358 showing positive staining. Heat mediated antigen retrieval was performed before commencing with IHC staining protocol.



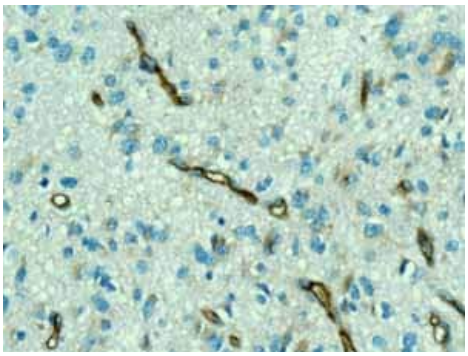
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PODXL antibody [EPR9518] (ab150358)

Immunohistochemical analysis of paraffin embedded human skeletal muscle tissue using unpurified ab150358 showing **negative staining**. Heat mediated antigen retrieval was performed before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PODXL antibody [EPR9518] (ab150358)

Immunohistochemical analysis of paraffin embedded normal human kidney tissue using unpurified ab150358 showing positive staining. Heat mediated antigen retrieval was performed before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PODXL antibody [EPR9518] (ab150358)

Immunohistochemical analysis of paraffin embedded human glioma tissue using unpurified ab150358 showing positive staining. Heat mediated antigen retrieval was performed before commencing with IHC staining protocol.

Anti-PODXL antibody [EPR9518] (ab150358)

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