

Anti-Podoplanin antibody [EPR22182] ab236529

Recombinant RabMAb

[6 References](#) [11 Images](#)

Overview

Product name	Anti-Podoplanin antibody [EPR22182]
Description	Rabbit monoclonal [EPR22182] to Podoplanin
Host species	Rabbit
Tested applications	Suitable for: WB, IHC-P, IP, Flow Cyt (Intra), Flow Cyt, mIHC
Species reactivity	Reacts with: Human
Immunogen	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: U-2 OS and U-87 MG whole cell lysates; Human seminoma, tonsil, lymph node and stomach lysates. IHC-P: Human colon, mesothelioma and Kaposi's sarcoma tissues. Flow Cyt (intra): U-87 MG and U-2 OS cells. IP: U-2 OS whole cell lysate. mIHC-P: Human lung tissue. Flow cyto: U-87 MG 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>

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 long term. Avoid freeze / thaw cycle.
Storage buffer	<p>pH: 7.2</p> <p>Preservative: 0.01% Sodium azide</p> <p>Constituents: PBS, 40% Glycerol, 0.05% BSA</p>
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR22182

Isotype

IgG

Applications

The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab236529 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
WB		1/1000. Detects a band of approximately 38 kDa (predicted molecular weight: 16 kDa).
IHC-P		1/4000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
IP		1/30.
Flow Cyt (Intra)		1/300.
Flow Cyt		Use at an assay dependent concentration.
mIHC		1/8000.

Target

Function

May be involved in cell migration and/or actin cytoskeleton organization. When expressed in keratinocytes, induces changes in cell morphology with transfected cells showing an elongated shape, numerous membrane protrusions, major reorganization of the actin cytoskeleton, increased motility and decreased cell adhesion. Required for normal lung cell proliferation and alveolus formation at birth. Induces platelet aggregation. Does not have any effect on folic acid or amino acid transport. Does not function as a water channel or as a regulator of aquaporin-type water channels.

Tissue specificity

Highly expressed in placenta, lung, skeletal muscle and brain. Weakly expressed in brain, kidney and liver. In placenta, expressed on the apical plasma membrane of endothelium. In lung, expressed in alveolar epithelium. Up-regulated in colorectal tumors and expressed in 25% of early oral squamous cell carcinomas.

Sequence similarities

Belongs to the podoplanin family.

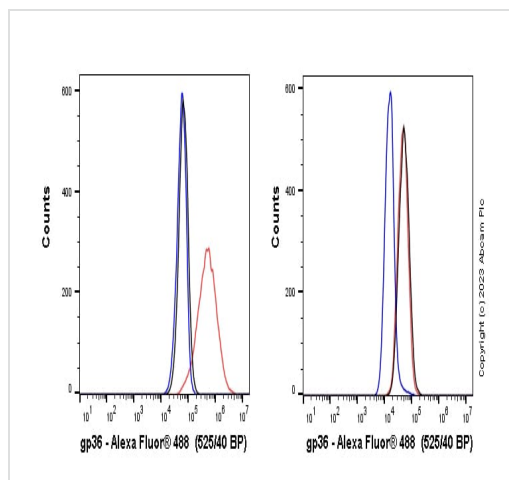
Post-translational modifications

Extensively O-glycosylated. Contains sialic acid residues. O-glycosylation is necessary for platelet aggregation activity.
The N-terminus is blocked.

Cellular localization

Membrane. Cell projection > filopodium membrane. Cell projection > lamellipodium membrane. Cell projection > microvillus membrane. Cell projection > ruffle membrane. Localized to actin-rich microvilli and plasma membrane projections such as filopodia, lamellipodia and ruffles.

Images

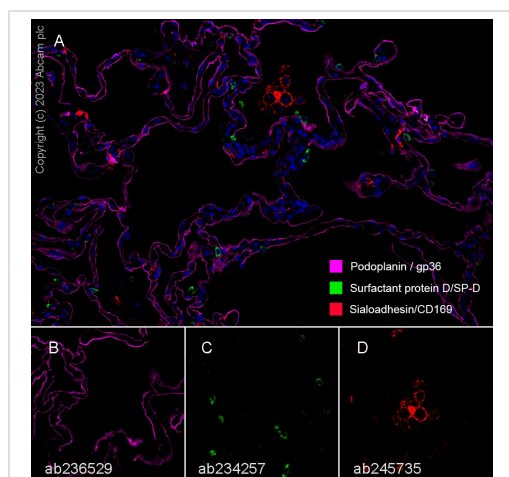


Flow Cytometry - Rabbit monoclonal [EPR22182] to Podoplanin (ab236529)

Flow cytometry overlay histogram showing left U-87 MG positive cells and right negative HUVEC stained with ab236529 (red line). The cells were incubated in 1x PBS containing 10% normal goat serum to block non-specific protein-protein interaction followed by the antibody (ab236529) (1×10^6 in 100 μ l at 1.0 μ g/ml (1/500)) for 30min on ice.

The secondary antibody Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed was incubated at 1/4000 for 30min on ice. Isotype control antibody (black line) was Recombinant Rabbit IgG, monoclonal [EPR25A] - Isotype Control used at the same concentration and conditions as the primary antibody. Unlabelled sample (blue line) was also used as a control.

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



Multiplex immunohistochemistry - Rabbit monoclonal [EPR22182] to Podoplanin (ab236529)

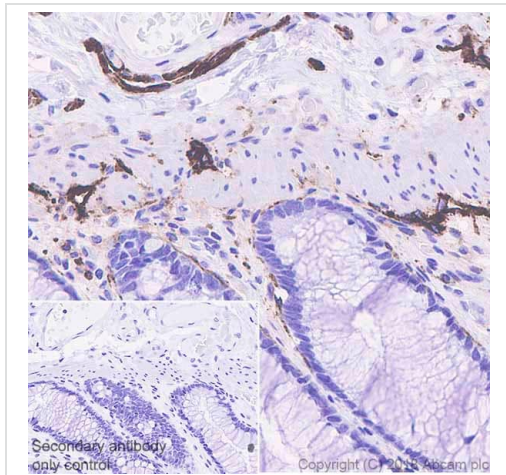
Fluorescence multiplex immunohistochemical analysis of formalin/PFA-fixed paraffin-embedded Human lung tissue. Panel A: Merged staining of anti-Podoplanin (magenta; Opal™690), anti-Surfactant protein D/SP-D (green; Opal™520) and anti-Sialoadhesin/CD169 (red; Opal™570) on human lung.

Panel B: Anti-Podoplanin stained on alveolar type I cells.

Panel C: Anti-Surfactant protein D/SP-D stained on alveolar type II cells.

Panel D: Anti-Sialoadhesin/CD169 stained on macrophages.

The section was incubated in three rounds of staining: in the order of ab236529 and **ab234257** for 30 mins, then **ab245735** for 10 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system. The immunostaining was performed on a Leica Biosystems BOND® RX instrument with an Opal™ 4-color kit. Image acquisition was performed with Leica SP8 confocal microscope. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20 mins. Counterstained with DAPI.

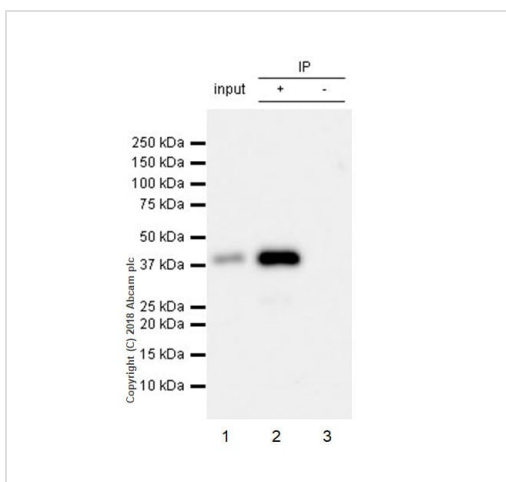


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Rabbit monoclonal [EPR22182] to Podoplanin (ab236529)

Immunohistochemical analysis of paraffin-embedded human colon tissue labeling Podoplanin with ab236529 at 1/4000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use. Positive staining in lymphatic endothelial cells of human colon (PMID: 28101903; PMID: 27798887) is observed. Counter stained with hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) Ready to use.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunoprecipitation - Rabbit monoclonal [EPR22182] to Podoplanin (ab236529)

Podoplanin was immunoprecipitated from 0.35 mg of U-2 OS (human bone osteosarcoma epithelial cell line) whole cell lysate with ab236529 at 1/30 dilution. Western blot was performed from the immunoprecipitate using ab236529 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) ([ab131366](#)), was used for detection at 1/5000 dilution.

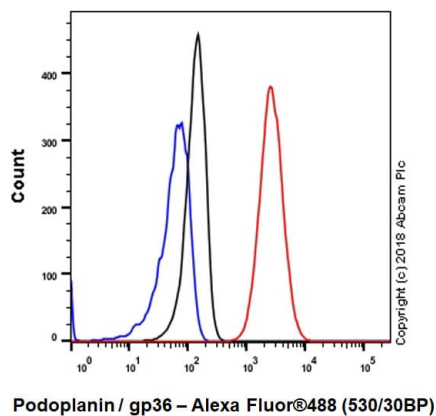
Lane 1: U-2 OS whole cell lysate 10 µg (Input).

Lane 2: ab236529 IP in U-2 OS whole cell lysate.

Lane 3: Rabbit monoclonal IgG ([ab172730](#)) instead of ab236529 in U-2 OS whole cell lysate.

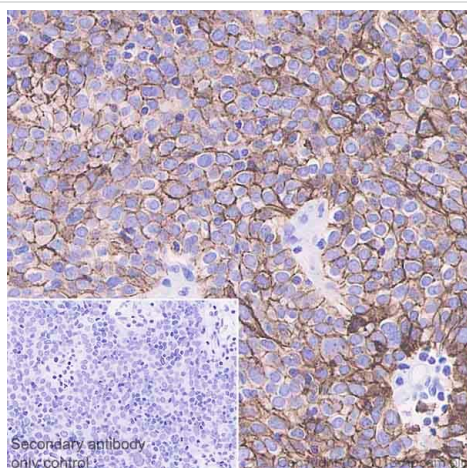
Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 30 seconds.



Flow Cytometry (Intracellular) - Rabbit monoclonal [EPR22182] to Podoplanin (ab236529)

Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed, 90% methanol-permeabilized U-2 OS (human bone osteosarcoma epithelial cell line) cell line labeling Podoplanin with ab236529 at 1/300 dilution (red) compared with a Rabbit IgG, monoclonal [EPR25A] - Isotype Control (**ab172730**) (black) and an unlabeled control (cells without incubation with primary antibody and secondary antibody) (blue). Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) at 1/2000 dilution was used as the secondary antibody.

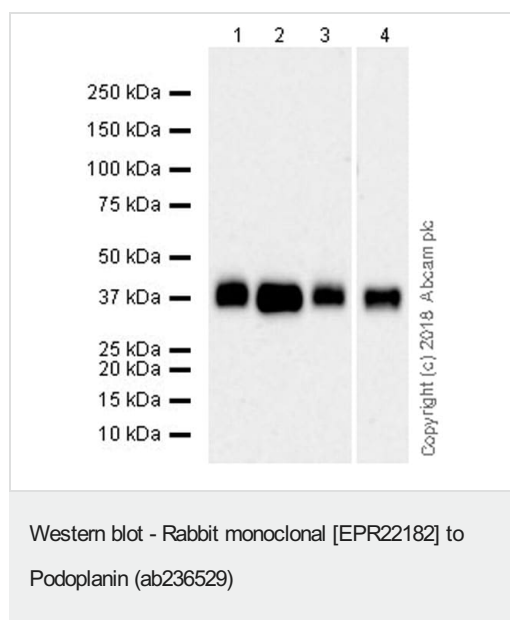


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Rabbit monoclonal [EPR22182] to Podoplanin (ab236529)

Immunohistochemical analysis of paraffin-embedded human mesothelioma tissue labeling Podoplanin with ab236529 at 1/4000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use. Membranous and weak cytoplasmic staining in tumor cells of human mesothelioma (PMID: 16670463) is observed. Counter stained with hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) Ready to use.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



All lanes : Anti-Podoplanin antibody [EPR22182] (ab236529) at 1/1000 dilution

Lane 1 : Human seminoma lysate

Lane 2 : Human tonsil lysate

Lane 3 : Human lymph node lysate

Lane 4 : Human stomach lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

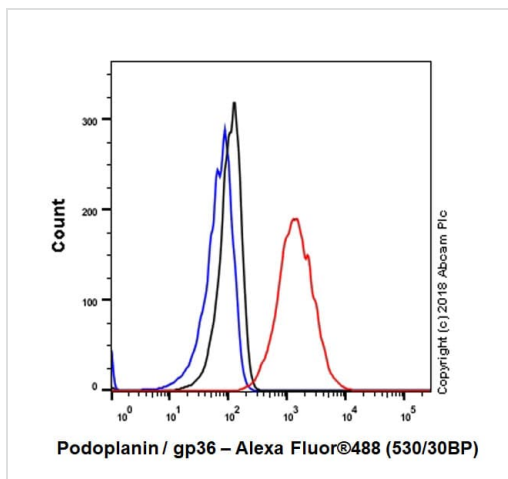
Predicted band size: 16 kDa

Observed band size: 38 kDa

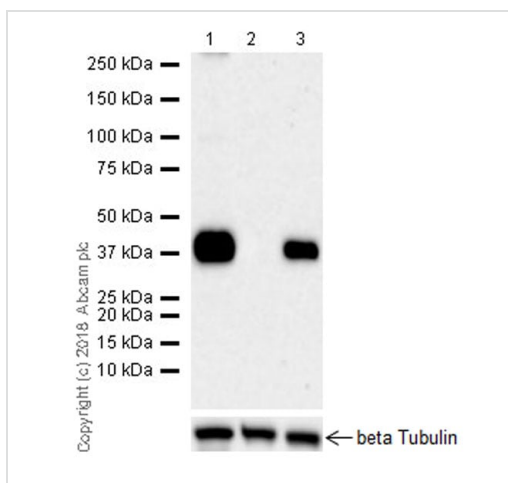
Exposure time: 3 minutes

Blocking/Dilution buffer: 5% NFDM/TBST.

The molecular mass observed is consistent with what has been described in the literature (PMID: 10408868; PMID: 17279584).



Flow Cytometry (Intracellular) - Rabbit monoclonal [EPR22182] to Podoplanin (ab236529)



Western blot - Rabbit monoclonal [EPR22182] to Podoplanin (ab236529)

Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed, 90% methanol-permeabilized U-87 MG (human glioblastoma-astrocytoma epithelial cell line) cell line labeling Podoplanin with ab236529 at 1/300 dilution (red) compared with a Rabbit IgG, monoclonal [EPR25A] - Isotype Control (**ab172730**) (black) and an unlabeled control (cells without incubation with primary antibody and secondary antibody) (blue). Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) at 1/2000 dilution was used as the secondary antibody.

All lanes : Anti-Podoplanin antibody [EPR22182] (ab236529) at 1/1000 dilution

Lane 1 : U-2 OS (human bone osteosarcoma epithelial cell line) whole cell lysate

Lane 2 : HUVEC (human umbilical vein endothelial cell line) whole cell lysate

Lane 3 : U-87 MG (human glioblastoma-astrocytoma epithelial cell line) whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

Predicted band size: 16 kDa

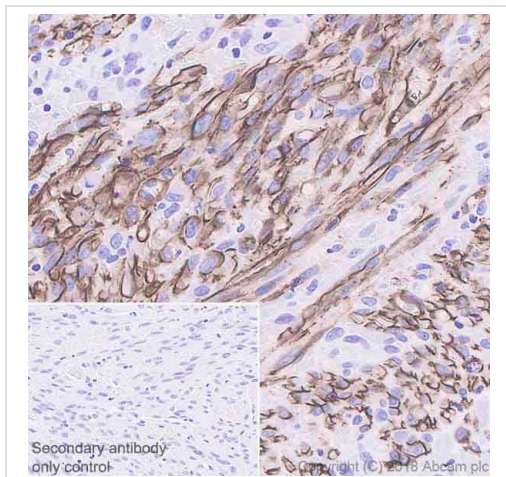
Observed band size: 38 kDa

Exposure time: 3 minutes

Blocking/Dilution buffer: 5% NFDm/TBST.

The molecular mass observed is consistent with what has been described in the literature (PMID: 10408868; PMID: 17279584).

Negative control: HUVEC (PMID: 21920451).







Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Rabbit monoclonal [EPR22182] to Podoplanin (ab236529)

Immunohistochemical analysis of paraffin-embedded human Kaposi's sarcoma tissue labeling Podoplanin with ab236529 at 1/4000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use. Membranous and cytoplasmic staining in tumor cells of human Kaposi's sarcoma (PMID: 11950918) is observed. Counter stained with hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) Ready to use.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Why choose a recombinant antibody?

 <p>Research with confidence Consistent and reproducible results</p>	 <p>Long-term and scalable supply Recombinant technology</p>
 <p>Success from the first experiment Confirmed specificity</p>	 <p>Ethical standards compliant Animal-free production</p>

Rabbit monoclonal [EPR22182] to Podoplanin (ab236529)

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