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Product datasheet for DM3500P

PDPN Mouse Monoclonal Antibody [Clone ID: 18H5]

Product data:

Product Type:	Primary Antibodies
Clone Name:	18H5
Applications:	FC, IF, IHC, WB
Recommended Dilution:	**FACS analysis** (1-10 µg/ml). **Immuofluorescence** (1-2 µg/ml). **Western blot analysis** (1-5 µg/ml). **Immunohistochemistry on Frozen and Paraffin Sections** (6-30 µg/ml).
Reactivity:	Human
Host:	Mouse
Isotype:	IgG1
Clonality:	Monoclonal
Immunogen:	GP36 (Podoplanin) expressed by MDCK cells.
Specificity:	This antibody reacts with Human Podoplanin (gp36). Other species not tested.
Formulation:	PBS, pH 7.4 without preservatives or stabilizers State: Aff - Purified State: Lyophilized purified IgG fraction from Cell Culture Supernatant
Reconstitution Method:	Restore in sterile water to a concentration of 0.1-1.0 mg/ml.
Purification:	Affinity Chromatography on Protein G
Conjugation:	Unconjugated
Storage:	Prior to reconstitution store at 2-8°C for one month. Following reconstitution store undiluted at 2-8°C for one month or (in aliquots) at -20°C for longer. Avoid repeated freezing and thawing.
Stability:	Shelf life: one year from despatch.
Gene Name:	podoplanin
Database Link:	Entrez Gene 10630 Human Q86YL7

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Background: Podoplanin, also known as glycoprotein 36 (gp36), PA2.26 antigen, T1alpha (T1A), and aggrus, is a 36 kDa type I transmembrane sialoglycoprotein and member of the Podoplanin family. Podoplanin has three potential splice variants, the longest of which is represented by a 238 amino acid (aa) precursor (NP_006465). It contains an undefined signal sequence, a 22 aa transmembrane segment (aa 207-228) and a short cytoplasmic tail (aa 229-238). The ECD contains abundant Ser/Thr residues that could serve as potential O-linked glycosolation sites. The cytoplasmic tail contains putative sites for protein kinase C phosphorylation. There are two potential alternate start sites at Met 77 (Swiss Prot #: Q86YL7) and Met 119 (EAW51692) that generate short forms. The 162 aa short form Podoplanin precursor shares 47% aa identity with mouse Podoplanin. Podoplanin is expressed on glomerular epithelial cells (podocytes), type I lung alveolar cells, lymphatic endothelial cells, and numerous tumors, including colorectal tumors, squamous cell carcinomas, testicular seminoma, and brain tumors. One study shows high expression of Podoplanin mRNA in placenta, lung, skeletal muscle, and heart, and weaker levels in brain, kidney, and liver. Podoplanin is the ligand for C-type lectin-like receptor 2 (CLEC2). Their association is dependent on sialic acid on O-glycans of Podoplanin. Through its association with CLEC2, Podoplanin-induces platelet aggregation and tumor metastasis. Podoplanin is also necessary for lymphatic vessel formation, normal lung cell proliferation and alveolus formation at birth.

Synonyms: Glycoprotein 36, PA2.26 antigen, T1-alpha, Aggrus, PDPN, GP36, PSEC0003, PSEC0025

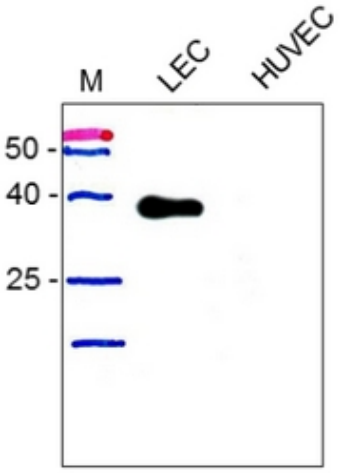
Note: Protocol: **Sample protocol for Formalin-Fixed Paraffin-Embedded Sections (as a guide only)**

1) Put Paraffin sections (2-4 µm) onto a Poly-Lysin object holder.
2) Deparaffination:
- Xylol (10 min).
- Acetone (10 min).
- Acetone / buffer mixture (10 min).
- Rinse 3x with wash buffer.
3) Microwave: boil up 3x 10 min in the appropriate buffer (put the whole glass curette into the microwave)
a) EDTA buffer: 0,1 M EDTA in aqua dest, pH 8,0.
b) Citrate buffer/10 mM, pH 6,0.
A: citric acid, 19,2 g in 1L aqua dest.
B: Na-Citrate, 29,41 g in 1L aqua dest.
9 ml stock solution A + 50 ml stock solution B, in 500 ml aqua dest (pH adjustment only in solution B)
Cool down the sections to RT for 25 min in wash buffer.
4) Addition of primary Abs (dilute e.g. in ready to use buffer from DAKO [S3022]).
- Shortly drip off the sections on paper (take 3-4 sections at once).
- Dust off the liquid beside the sections.
- Add primary Abs.
- incubate 0.5 h at RT.
- Rinse 3x with wash buffer.

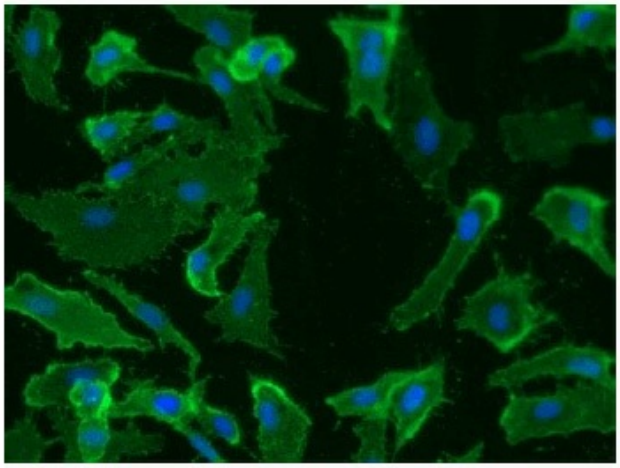
- Shortly drip off the sections.
5) Addition of the by-pass AB (monoclonal mouse-anti-rabbit IgG, e.g. DAKO [M0737] 1: 250)
- incubate 0.5 h at RT.
- Rinse 3x with wash buffer.
- Shortly drip off the sections.
6) Addition of anti-mouse-IgG from rabbit 1:
- Incubate 0.5 h at RT.
- Rinse 3x with wash buffer.
- Shortly drip off the sections.
7) Addition of the APAAP complexes.
- Incubate 0.5 h at RT.
- Rinse 3x with wash buffer.
- Shortly drip off the sections.
8) Addition of the Chromogens (NaphtolAS-MXPhosphat plus FastredTR-Salz).
- Incubate for 25 min at RT.
- Rinse 3x with wash buffer.
- Shortly drip off the sections.
- Counterstain: Hämalaun.
- 45 sec.
- rinse 3x 5 min in lukewarm tap water.
- rinse 1x 5 min in dest. H_2O.
9) Cover the sections in Glycergel (DAKO).
Note: For steps 6,7,8 use kit from DAKO: Universal-DAKO APAAP Kit Mouse.
Note: After each step the sections could stay in the buffer!

Wash buffer (for 40 L): 351g NaCl, 274g Tris-HCL, 36g Tris-Base.

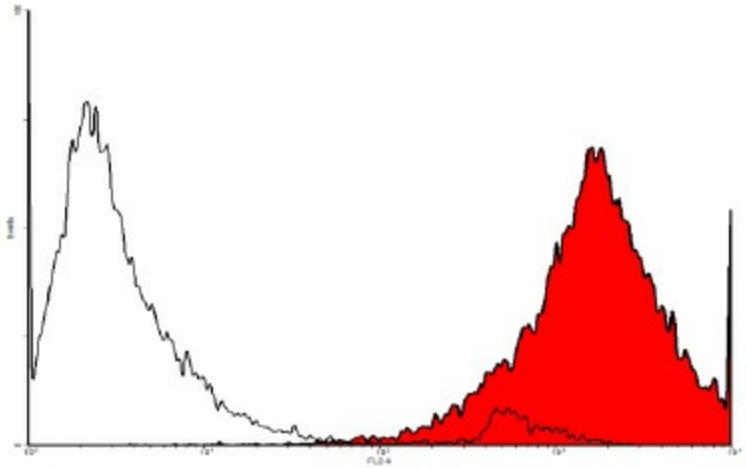
Product images:



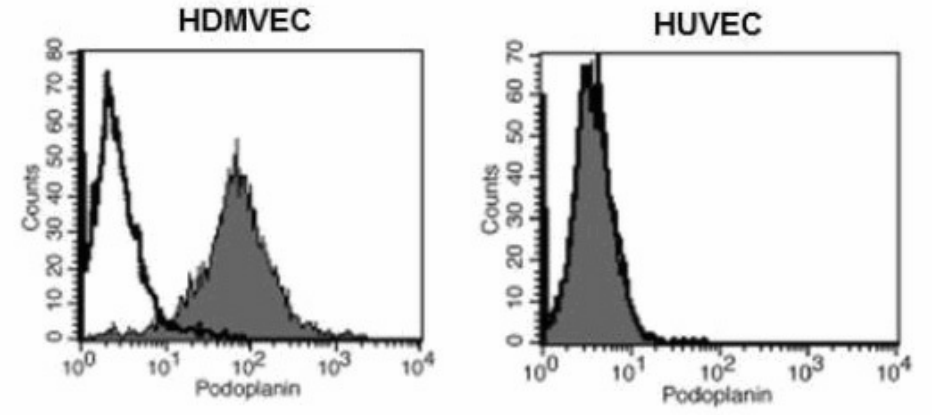
Western blot analysis of Podoplanin expression in Human lymphatic endothelial cells (LEC) and HUVECs. Total lysate of both cell types were subjected to SDS-PAGE and subsequent Western analysis with the Podoplanin Antibody. The antibody recognizes a protein of about 36 kDa in total lysate from LECs but not from HUVEC.



Immunofluorescence staining (green) of Podoplanin in primary human dermal lymphatic endothelial cells (HDLEC) with anti-Human Podoplanin Antibody V (0.5 ug/ml) and counter staining of nuclei with Dapi. As secondary antibody Goat anti-Mouse ALEXA Fluor 488 was used 1/400.



FACS analysis with Human primary lymphatic endothelial cells (HDLEC). As secondary antibody anti-Mouse IgG-PE was used.



FACS analysis with primary human dermal microvascular (HDMVEC) and umbilical vein (HUVEC) endothelial cells. The lymphatic endothelial cell marker Podoplanin is not expressed in the blood endothelial cells.