

DM3500P**Monoclonal Antibody to Podoplanin - Aff - Purified****Alternate names:**

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

Quantity:

0.1 mg

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 glycosylation 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.

Uniprot ID:[Q86YL7](#)**NCBI:**[NP_001006625.1](#)**GeneID:**[10630](#)**Host / Isotype:**

Mouse / IgG1

Clone:

18H5

Immunogen:

GP36 (Podoplanin) expressed by MDCK cells.

Format:**State:** Lyophilized purified IgG fraction from Cell Culture Supernatant**Purification:** Affinity Chromatography on Protein G**Buffer System:** PBS, pH 7.4 without preservatives or stabilizers**Reconstitution:** Restore in sterile water to a concentration of 0.1-1.0 mg/ml.**Applications:****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).

Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.

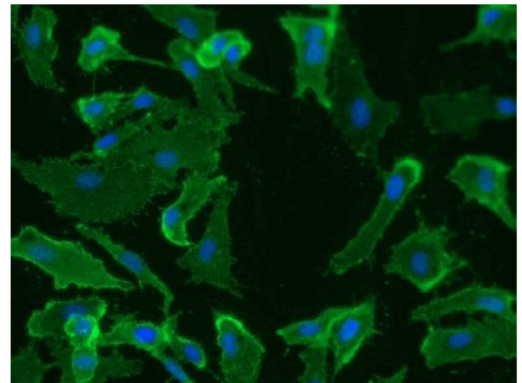
- Specificity:** This antibody reacts with Human Podoplanin (gp36). Other species not tested.
- Storage:** Prior to reconstitution store at 2-8°C for one month.
Following reconstitution store undiluted at 2-8°C for one month or (in aliquots) at -20°C for longer.
Avoid repeated freezing and thawing.
Shelf life: one year from despatch.
- Product Citations:** **Purchased from Acris:**
1. Kilic N, Oliveira-Ferrer L, Neshat-Vahid S, Irmak S, Obst-Pernberg K, Wurmbach JH, et al. Lymphatic reprogramming of microvascular endothelial cells by CEA-related cell adhesion molecule-1 via interaction with VEGFR-3 and Prox1. *Blood*. 2007 Dec 15;110(13):4223-33. Epub 2007 Aug 30. PubMed PMID: 17761831.
 2. Riesenberger R, Weiler C, Spring O, Eder M, Buchner A, Popp T, et al. Expression of indoleamine 2,3-dioxygenase in tumor endothelial cells correlates with long-term survival of patients with renal cell carcinoma. *Clin Cancer Res*. 2007 Dec 1;13(23):6993-7002. PubMed PMID: 18056175.
 3. Conrad C, Niess H, Huss R, Huber S, von Luettichau I, Nelson PJ, et al. Multipotent mesenchymal stem cells acquire a lymphendothelial phenotype and enhance lymphatic regeneration in vivo. *Circulation*. 2009 Jan 20;119(2):281-9. doi: 10.1161/CIRCULATIONAHA.108.793208. Epub 2008 Dec 31. PubMed PMID: 19118255.
 4. Turner JD, Langley RS, Johnston KL, Gentil K, Ford L, Wu B, et al. Wolbachia lipoprotein stimulates innate and adaptive immunity through Toll-like receptors 2 and 6 to induce disease manifestations of filariasis. *J Biol Chem*. 2009 Aug 14;284(33):22364-78. doi: 10.1074/jbc.M901528200. Epub 2009 May 19. PubMed PMID: 19458089.
 5. Birke K, Lütjen-Drecoll E, Kerjaschki D, Birke MT. Expression of podoplanin and other lymphatic markers in the human anterior eye segment. *Invest Ophthalmol Vis Sci*. 2010 Jan;51(1):344-54. doi: 10.1167/iovs.08-3307. Epub 2009 Sep 8. PubMed PMID: 19737877.
- General Readings:**
1. Breiteneder-Geleff S, Matsui K, Soleiman A, Meraner P, Poczewski H, Kalt R, et al. Podoplanin, novel 43-kd membrane protein of glomerular epithelial cells, is down-regulated in puromycin nephrosis. *Am J Pathol*. 1997 Oct;151(4):1141-52. PubMed PMID: 9327748.
 2. Zimmer G, Oeffner F, Von Messling V, Tschernig T, Grönness HJ, Klenk HD, et al. Cloning and characterization of gp36, a human mucin-type glycoprotein preferentially expressed in vascular endothelium. *Biochem J*. 1999 Jul 15;341 (Pt 2):277-84. PubMed PMID: 10393083.
 3. Schacht V, Ramirez MI, Hong YK, Hirakawa S, Feng D, Harvey N, et al. T1alpha/podoplanin deficiency disrupts normal lymphatic vasculature formation and causes lymphedema. *EMBO J*. 2003 Jul 15;22(14):3546-56. PubMed PMID: 12853470.
- Protocols:** **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. H₂O.
 - 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!

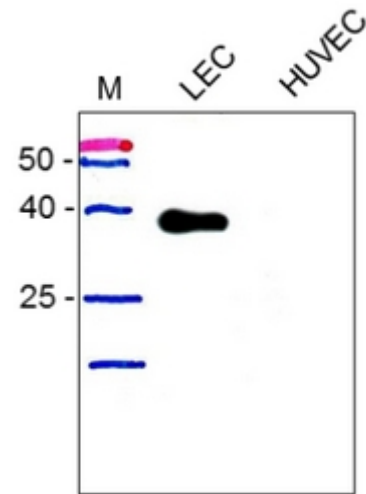
Wash buffer (for 40 L): 351g NaCl, 274g Tris-HCL, 36g Tris-Base.

Pictures:

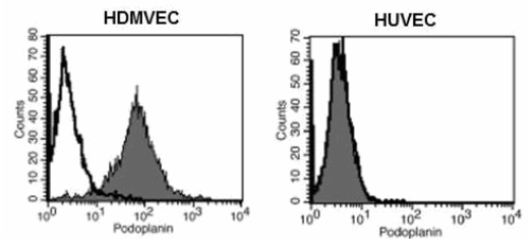
Immunofluorescence staining (green) of Podoplanin in primary human dermal lymphatic endothelial cells (HDLEC) with anti-Human Podoplanin Antibody VCat.-No DM3500P (0.5 µg/ml) and counter staining of nuclei with Dapi. As secondary antibody Goat anti-Mouse ALEXA Fluor 488 was used 1/400.



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 Cat.-No DM3500P. The antibody recognizes a protein of about 36 kDa in total lysate from LECs but not from HUVEC.



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.



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

