AM32708PU-N

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Monoclonal Antibody to CD31 / PECAM1 - Purified

Alternate names: EndoCAM, GPIIA', PECAM-1, Platelet endothelial cell adhesion molecule

Catalog No.: AM32708PU-N

Quantity: 0.1 mg

Concentration: 0.5 mg/ml

Background: Platelet endothelial cell adhesion molecule-1 (PECAM-1) or cluster designation 31 (CD31) is a member of the immunoglobulin (Ig) gene superfamily and a transmembrane glycoprotein. The CD31 protein is widely expressed by cells of the vascular endothelium lineage, platelets, Kupffer cells, granulocytes, megakaryocytes, monocytes, neutrophils, and some types of T-cells. CD31 functions as a cell adhesion molecule in endothelial cell homotypic interactions in the process of angiogenesis. CD31 is also involved in leukocyte migration and integrin activation. It plays a key role in the adhesion cascade between the endothelial cells and inflammatory cells, enabling leukocyte migration in inflammatory sites. CD31 is one of the best markers for benign and malignant vascular tumors. It is expressed in certain tumors including angiomas and angiosarcomas including epitheloid hemangioendothelioma, epitheloid sarcoma-like hemangioendothelioma. Its expression in Kaposi sarcoma lesions is variable. Human CD31 comprises 738 amino acids and its predicted molecular weight would therefore be approximately 81kDa. However the observed molecular weight, as reported by various research groups including our own, is higher and at around 120kDa and 140kDa. The disparity probably reflects post translational modifications including glycosylation and probably because different isoforms of the protein may exist.

Uniprot ID: P16284

NCBI: 9606

GeneID: 5175

Host / Isotype: Mouse / IgG1

Recommended Isotype Controls: SM10P (for use in human samples), SM20P (for use in rat samples), AM03095PU-N

Clone: BC16-6.4EF

Immunogen: Biochemically characterized crude subcellular/membrane fraction obtained from HUVEC cells.

Format:
State: Liquid purified IgG fraction from Tissue Culture Supernatant
Purification: Protein G Sepharose Chromatography
Buffer System: PBS, pH 7.2
Preservatives: 0.05% Sodium Azide

Applications: **Western Blot:** Use the *BC16-6.4EF* antibody at 2-3 µg/ml in Tris buffered Saline with 0.05% Tween 20 and 5% non-fat dry milk (Blotto) or similar diluents. The antibody reacts with a

band of Approximate Molecular size 135kDa.
Immunostaining: Use the *BC16-6.4EF* antibody at 2-3 µg/ml diluted with PBS containing 1% BSA.
Indirect Immunofluorescence.
Immunohistochemistry: 3-5 µg/ml.
The *BC16-6.4EF* antibody may be used on endothelial cells such as HUVEC cells grown on chamber slides, cytospins and cryosections of Human tissue.
For staining, the cultured cells and cryosections should be fixed in 1-2% paraformaldehyde for 30 mins, permeabilized in 0.25% Triton X 100 in PBS for 30 mins and non-specific binding blocked with 1% BSA in PBS.
The primary antibody may be diluted in PBS with 1% BSA and incubated on cells/tissue overnight at 4°C.
The antibody may also be used to inhibit endothelial cell–cell interactions.
Suggested Positive Control Cells and Tissues: For staining and Western blotting, Human Umbilical Endothelial Cells (Thermo Fisher). For immunohistochemical staining, cryosections of most Human tissue with vasculature may be used.
Immunohistochemistry on Paraffin Sections: 2-5 µg/ml.
Heat induced antigen retrieval with citrat buffer, pH 6.2 using a pressure cooker was preformed. Sections were blocking using a commercially available casein solution. Signal was generated using a commercially available polymer HRP detection system and DAB.
Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.

Molecular Weight: Approximately 81 kDa (Predicted), a higher band of about 135kDa is observed. The higher Molecular Weight observed may be due post translational modifications including glycosylation. A significantly weaker band of Approximately 55kDa is also observed.

Specificity: The antibody was tested against normal and Human tumor tissue and HUVEC cells.
The antibody was also tested against Mouse and Rat tissues. Staining of vasculature is observed in all tissues examined.
The HUVEC cells showed cell surface and cytoplasmic staining. Not tested on Formalin Fixed, Paraffin Embedded (FFPE) tissue.

Species Reactivity: **Tested:** Human, Mouse, Rat.

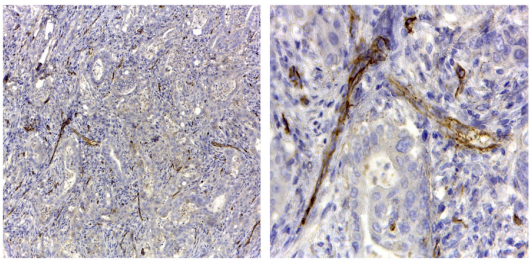
Storage: Store undiluted at 2-8°C for one month or (in aliquots) at -20°C for longer.
Avoid repeated freezing and thawing.
Shelf life: one year from despatch.

General Readings:
1. Hsu-Lin S, Berman CL, Furie BC, August D, Furie B. A platelet membrane protein expressed during platelet activation and secretion. Studies using a monoclonal antibody specific for thrombin-activated platelets. J Biol Chem. 1984 Jul 25;259(14):9121-6. PubMed PMID: 6746643.
2. van Mourik JA, Leeksma OC, Reinders JH, de Groot PG, Zandbergen-Spaargaren J. Vascular endothelial cells synthesize a plasma membrane protein indistinguishable from the platelet membrane glycoprotein IIa. J Biol Chem. 1985 Sep 15;260(20):11300-6. PubMed PMID: 3161894.
3. Newman PJ, Berndt MC, Gorski J, White GC, Lyman S, Paddock C, et al. PECAM-1 (CD31) cloning and relation to adhesion molecules of the immunoglobulin gene superfamily. Science. 1990 Mar 9;247(4947):1219-22. PubMed PMID: 1690453.
4. Simmons DL, Walker C, Power C, Pigott R. Molecular cloning of CD31, a putative intercellular adhesion molecule closely related to carcinoembryonic antigen. J Exp Med. 1990 Jun 1;171(6):2147-52. PubMed PMID: 2351935.
5. Stockinger H, Gadd SJ, Eher R, Majdic O, Schreiber W, Kasinrerk W, et al. Molecular characterization and functional analysis of the leukocyte surface protein CD31. J Immunol. 1990 Dec 1;145(11):3889-97. PubMed PMID: 1700999.

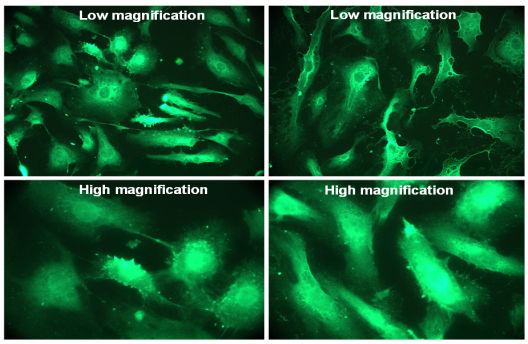
6. Albelda SM, Oliver PD, Romer LH, Buck CA. EndoCAM: a novel endothelial cell-cell adhesion molecule. J Cell Biol. 1990 Apr;110(4):1227-37. PubMed PMID: 2182647.
7. Albelda SM, Muller WA, Buck CA, Newman PJ. Molecular and cellular properties of PECAM-1 (endoCAM/CD31): a novel vascular cell-cell adhesion molecule. J Cell Biol. 1991 Sep;114(5):1059-68. PubMed PMID: 1874786.
8. Muller WA, Weigl SA, Deng X, Phillips DM. PECAM-1 is required for transendothelial migration of leukocytes. J Exp Med. 1993 Aug 1;178(2):449-60. PubMed PMID: 8340753.
9. Vaporciyan AA, DeLisser HM, Yan HC, Mendiguren II, Thom SR, Jones ML, et al. Involvement of platelet-endothelial cell adhesion molecule-1 in neutrophil recruitment in vivo. Science. 1993 Dec 3;262(5139):1580-2. PubMed PMID: 8248808.
10. Kirschbaum NE, Gumina RJ, Newman PJ. Organization of the gene for human platelet/endothelial cell adhesion molecule-1 shows alternatively spliced isoforms and a functionally complex cytoplasmic domain. Blood. 1994 Dec 15;84(12):4028-37. PubMed PMID: 7994021.
11. Miettinen M, Lindenmayer AE, Chaubal A. Endothelial cell markers CD31, CD34, and BNH9 antibody to H- and Y-antigens--evaluation of their specificity and sensitivity in the diagnosis of vascular tumors and comparison with von Willebrand factor. Mod Pathol. 1994 Jan;7(1):82-90. PubMed PMID: 7512718.
12. Pantanowitz L, Moses AV, Früh K. CD31 immunohistochemical staining in Kaposi Sarcoma. Arch Pathol Lab Med. 2012 Nov;136(11):1329; author reply 1330. doi: 10.5858/arpa.2012-0153-LE. PubMed PMID: 23106575.

Pictures:

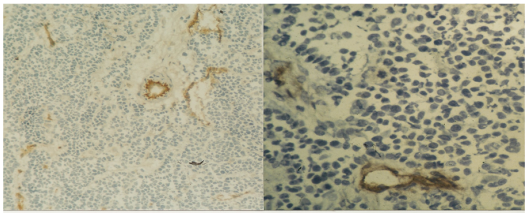
Staining of FFPE Human colon cancer (10x and 40x) with CD31/PECAM1 Antibody Cat.-No AM32708PU-N (Clone BC16-6.4EF) at 2 µg/ml. Antibody positive in cytoplasm of interstitial endotelial cells modulating neoangiogenesis.



Human Umbilical Vein Endothelial Cells (HUVEC) stained with 5 µg/ml CD31/PECAM1 Antibody Cat.-No AM32708PU-N (Clone BC16-6.4EF) diluted in PBS with 1% BSA. FITC conjugated Rabbit anti Mouse Ig secondary.



CD31/PECAM1 Antibody Cat.-No AM32708PU-N (Clone BC16-6.4EF) staining of the vasculature in small round cell tumors. Indirect immunoperoxidase staining with Peroxidase conjugated anti-Mouse Ig secondary antibody. DAB substrate, hematoxylin counterstain.



Western blotting with anti CD31/PECAM1 Antibody Cat.-No AM32708PU-N (Clone BC16-6.4EF) used at 2 µg/ml (in Blotto) on HUVEC cell lysate loaded at 10, 5 and 2.5 µg/lane.
Secondary Antibody: Peroxidase conjugated Rabbit anti-Mouse Ig used at 1/10,000 dilution in Blotto.
Developed by enhanced chemiluminescence.