Monoclonal Mouse Anti-Human CD31, Endothelial Cell Clone JC70A
Code No. M 0823 Lot 042. Edition 09.04.02

Intended use
For in vitro diagnostic use. DAKO Monoclonal Mouse Anti-Human CD31, Endothelial Cell, Clone JC70A, is intended for use in immunocytochemistry. The antibody primarily labels endothelial cells, and is a useful tool for the identification of benign and malignant vascular disorders, including angiosarcomas (1, 2). In addition, the antibody is valuable for the labelling of vessels when determining angiogenesis in several types of tumours (3-5). Differential identification is aided by the results from a panel of antibodies. Interpretation must be made within the context of the patient’s clinical history and other diagnostic tests by a qualified pathologist.

Synonym for antigen
PECAM-1 (platelet/endothelial cell adhesion molecule-1) (6).

Introduction
CD31 is a single chain type-1 transmembrane protein with a molecular mass of approximately 135 kDa, belonging to the immunoglobulin superfamily. In human serum, alternatively spliced versions of CD31 have been detected, including a form apparently lacking a transmembrane domain, but including the cytoplasmic tail (6).

CD31 binds in both a homophilic and heterophilic manner. The heterophilic ligands include heparan sulfate glycosaminoglycans, heparin, and the integrin αvβ3. CD31 plays a role in adhesive interactions between adjacent endothelial cells as well as between leucocytes and endothelial cells. The ligation of CD31 to the surface of leucocytes, results in upregulation of the functional leucocyte integrins. During inflammation, the leucocyte diapedesis across the endothelium involves homophilic CD31 interactions. In addition, heterophilic CD31 interaction has a separate role in the migration of monocytes across the subendothelial basal lamina (6).

CD31 is expressed on all continuous endothelia, including those of arteries, arterioles, venules, veins, and non-sinusoidal capillaries, but it is not expressed on discontinuous endothelium in e.g. splenic red pulp. In addition, CD31 is expressed diffusely on the surface of megakaryocytes, platelets, myeloid cells, natural killer cells, and some subsets of T cells, as well as on B-cell precursors (6).

Reagent provided
Monoclonal mouse antibody provided in liquid form as cell culture supernatant dialysed against 0.05 mol/L Tris/HCl, pH 7.2, and containing 15 mmol/L NaN3. Clone: JC70A (1). Isotype: IgG1, kappa. Mouse IgG concentration: 450 mg/L. Total protein concentration: 15.1 g/L.

Immunogen
Cell membrane preparation from the spleen of a patient with hairy cell leukaemia (1).

Specificity
The antibody was clustered as anti-CD31 at the Fifth International Workshop and Conference on Human Leucocyte Differentiation Antigens held in Boston in 1993 (7). The epitope recognized was found to be within the extracellular domain 1 (6).

In Western blotting of membrane preparations from a spleen rich in the antigen or from normal platelets, the antibody labels bands of respectively 100 kDa and 130 kDa, the latter corresponding to classic CD31. The smaller band of 100 kDa observed with the splenic preparation may be due to proteolytic breakdown or to variations in glycosylation (1).

Precautions
1. For in vitro diagnostic use.
2. This product contains sodium azide (NaN3), a chemical highly toxic in pure form. At product concentrations, not classified as hazardous, sodium azide may react with lead and copper plumbing to form highly explosive build-ups of metal azides. Upon disposal, flush with large volumes of water to prevent metal azide build-up in plumbing.

Storage
Store at 2-8 °C. Do not use after expiration date stamped on vial. If reagents are stored under any conditions other than those specified, the user must verify the conditions. There are no obvious signs to indicate instability of this product. Therefore, positive and negative controls should be run simultaneously with patient specimens. If unexpected staining is observed which cannot be explained by variations in laboratory procedures and a problem with the antibody is suspected, contact DAKO Technical Services.

Specimen preparation
Paraffin sections: The antibody can be used for labelling paraffin-embedded tissue sections fixed in formalin. Pre-treatment of tissues with proteinase K or heat-induced epitope retrieval is required. For heat-induced epitope retrieval, the following solutions are recommended: 10 mmol/L Tris buffer, 1 mmol/L EDTA, pH 9.0; DAKO Target Retrieval Solution, code No. S 1700; and DAKO Target Retrieval Solution, High pH, code No. S 3308. 10 mmol/L citrate buffer, pH 6.0, and pre-treatment of tissues with proteinase K were less efficient. The tissue sections should not dry out during the treatment or during the following immunocytochemical staining procedure.
Frozen sections and cell preparations: The antibody can be used for labelling frozen sections and cell preparations (1).

Staining procedure

Dilution: DAKO Monoclonal Mouse Anti-Human CD31, Endothelial Cell, code No. M 0823, may be used at a dilution range of 1:20-1:40 when applied on formalin-fixed, paraffin-embedded sections of human tonsil and using 15 minutes heat-induced epitope retrieval in 10 mmol/L Tris buffer, 1 mmol/L EDTA, pH 9.0, and 30 minutes incubation at room temperature with the primary antibody. Optimal conditions may vary depending on specimen and preparation method, and should be determined by each individual laboratory. The recommended negative control is DAKO Mouse IgG1, code No. X 0931, diluted to the same mouse IgG concentration as the primary antibody.

Visualization: DAKO LSAB®+/HRP kit, and DAKO EnVision™+/HRP kits, code Nos. K 4004 and K 4006, are recommended. For frozen sections and cell preparations, the DAKO APAAP kit, code No. K 0670, is a good alternative if endogenous peroxidase staining is a concern. Follow the procedure enclosed with the selected visualization kit.

Performance characteristics

Cells labelled by the antibody predominantly display staining of the cell membrane, with weaker cytoplasmic staining.

Normal tissues: The antibody labels endothelial cells in a wide range of tissues, including endothelium in renal glomerular capillaries and the endothelium of vasa vasorum. In addition the antibody labels megakaryocytes and occasional plasma cells in bone marrow (1). In frozen sections of human tonsil and spleen the antibody labels some non-endothelial cells, including some mantle zone B cells and T cells, and in blood smears the antibody labels neutrophil polymorphs, 50% of the lymphocytes, all of the monocytes, and platelets (1).

Abnormal tissues: The antibody labels endothelial cells in a variety of benign and malignant vascular lesions. In 10/10 (1) and 6/7 (2) angiosarcomas, respectively, the antibody labelled malignant vascular endothelial cells. Further, the antibody labelled 17/17 (2) and 3/3 (1) hemangiomas, respectively, 3/3 epithelioid hemangiomas, 1/1 papillary endovascular angioendothelioma (2), 2/2 angiofibromas, 1/1 hemangioendothelioma, 1/1 chemodectoma, 3/3 atrial myxomas and 2/2 cystic hygromas (1). In addition, the antibody labelled endothelial cells in tumour tissues with angiogenesis (3-5).

In lymphangiomas discrepant results have been observed as the antibody was reported to label 8/8 (2) and 0/4 (1) cases, respectively. Likewise for glomus tumours, where 2/2 (1) and 0/7 (2) cases were labelled by the antibody. No labelling was observed in one case each of lymphoepithelial cyst and pneumatosi coli (1), negative were also all of 30 benign, and 4 malignant nerve sheath tumours, 11 dermatofibromas, 28 dermatofibrosarcoma protuberans, 6 leiomysarcomas, 3 leiomyosarcomas, 3 giant cell fibroblastomas (2), 52 rhabdomyosarcomas, 16 small round cell tumours, 11 neuroblastomas, 23 Wilms’ tumours, 20 retinoblastomas, 13 benign, 7 small noncleaved cell malignant lymphomas. Additionally, spindle cells in 17 cases of Kaposi’s sarcomas were uniformly negative (8).

References


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