DATA SHEET

Monoclonal Mouse Anti-Human CD31, Endothelial Cell,

Clone JC70A

Catalog number: M082329

Intended for use in immunohistochemistry.

Intended use For in vitro diagnostic use.

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. The clinical interpretation of any staining or its absence should be complemented by morphological studies using proper controls and should be evaluated 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). Summary and explanation 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 surfaces of leucocytes results in upregulation of the functional leucocyte integrins, and 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 surfaces 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: see label on vial. The protein concentration between lots may vary without influencing the optimal dilution. The titer of each individual lot is compared and adjusted to a reference lot to ensure a consistent immunohistochemical staining performance from lot-to-lot.

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 (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

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1. For professional users.

2. This product contains sodium azide (NaN3), a chemical highly toxic in pure form. At product concentrations, though 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.

3. As with any product derived from biological sources, proper handling procedures should be used.

4. Wear appropriate Personal Protective Equipment to avoid contact with eyes and skin.

5. Unused solution should be disposed of according to local, State and Federal regulations.

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 conditions must be verified by the user. 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 heat-induced epitope retrieval is required. Optimal results are obtained with Dako Target Retrieval Solution, code S1700, Dako Target Retrieval Solution, High pH, code S3308, or 10 mmol/L Tris buffer, 1 mmol/L EDTA, pH 9.0. Less optimal results are obtained with 10 mmol/L citrate buffer, pH 6.0 and pre-treatment of tissues with proteinase K. The tissue sections should not dry out during the treatment or during the following immunohistochemical staining procedure.

Frozen sections and cell preparations: The antibody can be used for labelling frozen sections and cell preparations (1).

Staining procedure Dilution: Monoclonal Mouse Anti-Human CD31, Endothelial Cell, code M0823, 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 20 minutes heat-induced epitope retrieval in Dako Target Retrieval solution, code S1700, 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 X0931, diluted to the same mouse IgG concentration as the primary antibody. Unless the stability of the diluted antibody and negative control has been established in the actual staining procedure, it is recommended to dilute these reagents immediately before use, or dilute in Dako Antibody Diluent, code S0809. Positive and negative controls should be run simultaneously with patient specimen.

Visualization: Dako LSAB™+/HRP kit, code K0679, and Dako EnVision™+/HRP kits, codes. K4004 and K4006, 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. 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), 3/3 angiofibromas, 2/2 angiokeratomas, 1/1 hemangiopericytoma, 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 pneumatosis coli (1), negative were also all of 30 benign, and 4

malignant nerve sheath tumours, 11 dermatofibromas, 28 dermatofibrosarcoma protuberans, 6 leiomyomas, 3 leiomyosarcomas, 3 giant cell fibroblastomas (2), 52 rhabdomyosarcomas, 16 small round cell tumours, 11 neuroblastomas, 23 Wilms' tumours, 20 retinoblastomas, 13 esthesioneuroblastomas, and 7 small noncleaved cell malignant lymphomas. Additionally, spindle cells in 17 cases of Kaposi's sarcomas were uniformly negative (8).

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