Monoclonal Mouse Anti-Human CD21
Clone 1F8
Code No. M 0784
Lot 082. Edition 16.09.02

Intended use
For in vitro diagnostic use.
DAKO Monoclonal Mouse Anti-Human CD21, Clone 1F8, is intended for use in immunocytochemistry. The antibody labels follicular dendritic cells and mature B cells and it is a useful tool for the identification of structural alterations in the meshwork of follicular dendritic cells, which are frequently found in malignant lymphomas (1). 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.

Synonyms for antigen
C3d-receptor, CR2, EBV-receptor (2).

Introduction
CD21 is a transmembrane glycoprotein belonging to a family of complement regulatory proteins, comprising CD35, C4-binding protein, factor H and CD55. CD21 has an Mr of 145 000 and in its soluble form, sCD21, an Mr of 130 000. The extracellular region consists of multiple short consensus repeats, which contain several distinct ligand-binding sites. Binding of the specific ligands results in differential transmembrane signalling via the cytoplasmic tail that has potential protein kinase C and tyrosine kinase phosphorylation sites (2, 3).

CD21 is expressed by follicular dendritic cells (FDCs) and mature B cells (3). FDCs form a three-dimensional meshwork in B-cell follicles, which apparently defines the structure of the follicular compartment (1). On lymphoid tissue B cells, CD21 expression is strong on marginal zone, and moderate on mantle zone B cells. Germinal centre B cells have been found to be negative with most CD21 mAbs, and bone marrow B cells have little or no CD21 expression. The expression of CD21 is gradually lost, together with IgD, after stimulation of resting B cells in vitro. Further, CD21 has been found on several types of epithelial cells, and with low expression on T-cell acute lymphoblastic leukaemic cells, subsets of normal thymocytes, and mature T cells (3).

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: 1F8 (4). Isotype: IgG1, kappa.
Mouse IgG concentration: 350 mg/L. Total protein concentration: 8.0 g/L.

Immunogen
CD21 preparation purified from human tonsils to 60% purity (4).

Specificity
In Western blotting of the immunogen, the antibody labels a band of 145 kDa, corresponding to CD21. The epitope recognized by the antibody is located within a 72 kDa C3d-binding fragment (4).

The antibody labels cells or cell lines known to express CD21 (Raji, NC 37, tonsil cells), whereas no labelling is observed in the CD21-negative JURKAT cells (T-cell line) and human erythrocytes (4).

Precautions
1. For in vitro diagnostic use.
2. The NaN3 used as a preservative is toxic if ingested. NaN3 may react with lead and copper plumbing to form highly explosive metal compounds. Upon disposal, flush with large volumes of water to prevent 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 heat-induced epitope retrieval is required. Alternatively, protease K may be used, but it is considered to produce less consistent results. For heat-induced epitope retrieval, DAKO Target Retrieval Solution, code No. S 1700, provides optimal results. Heat-induced epitope retrieval in 10 mmol/L Tris buffer, pH 6.0; 10 mmol/L EDTA, pH 9.0; or Target Retrieval Solution, High pH, code No. S 3308, was found less efficient or destructive for the epitope. 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 acetone-fixed, frozen sections.
**Staining procedure**

**Dilution:** DAKO Monoclonal Mouse Anti-Human CD21, code No. M 0784, may be used at a dilution range of 1:25-1:50 when applied on formalin-fixed, paraffin-embedded sections of human tonsil and using 15 minutes heat-induced epitope retrieval in DAKO Target Retrieval Solution, code No. S 1700, 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. As negative control, DAKO Mouse IgG1, code No. X 0931, diluted to the same mouse IgG concentration as the primary antibody, is recommended.

**Visualization:** DAKO LSAB®+/HRP kit, code No. K 0679, 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 display staining confined to the cell membrane.

**Normal tissues:** In reactive lymphoid hyperplasia, the antibody strongly labels germinal centre FDCs in 11/11 cases, with a densely meshed FDC staining of the light zone and a loosely arranged, much less compact FDC staining of the dark zone. The antibody labels plasma cells, and gives a faint and less consistent labelling of lymphocytes in the mantle zone. Sinus-lining cells and mononcytoid B cells are weakly labelled (1).

**Abnormal tissues:** In 6 cases of B-cell chronic lymphocytic leukaemia, the antibody showed weak surface labelling of neoplastic cells in four cases, and labelled FDCs in the sparse residual germinal centres in four cases. In 8/8 mantle cell lymphomas, the antibody labelled loosely arranged, ill-defined, and expanded FDC meshworks of either nodular or diffuse patterns, resembling broken-up primary follicles. In 11/11 follicular lymphomas, the abnormal follicles demonstrated dense, sharply defined, expanded and sometimes merging FDC meshworks. However, only loose and patchy FDC labelling was observed in five cases with high-grade transformation in the areas of diffuse large cell lymphoma. In 7/7 low-grade MALT-type B-cell lymphomas, the antibody labelled expanded FDC meshworks, with particularly dense and confluent appearance in cases of primary salivary gland and gastric lymphomas. In 5/5 T-cell and histioocyte-rich B-cell lymphomas, the antibody labelled a few compressed residual follicles at the periphery of the lymphomatous involvement. In 9/9 angioimmunoblastic T-cell lymphomas, clusters of dendritic cells with FDC morphology appeared with frequent incorporation of proliferating postcapillary venules. In 4/4 nodular lymphocyte predominance Hodgkin’s lymphomas, the antibody labelled enlarged FDC meshworks overlapping the expanded mantle zones. In 15 cases of Hodgkin’s lymphoma of nodular sclerosis subtype, the antibody labelled sharply defined, sometimes irregular FDC meshworks surrounding the negative tumour cells in 11 cases of grade I, and showed sparse or no labelling in 4 cases of grade II disease (1). In follicular dendritic sarcoma, the antibody labelled neoplastic cells in 17/17 cases (5). In another study (6) the antibody labelled Reed-Sternberg and Hodgkin’s cells in 7/37 cases of nodular sclerosing, 2/41 cases of mixed cellularity, and 5/12 cases of lymphocyte depletion Hodgkin’s lymphoma, whereas no labelling of tumour cells was observed in 4 cases of lymphocyte predominance type. In nine of the cases where Reed-Sternberg and Hodgkin’s cells were labelled by the antibody, the cells expressed no other B- or T-cell associated markers (6).

**References**


