Monoclonal Mouse
Anti-Human CD20cy, B Cell
Clone L26
Code No. M 0755
Lot 072. Edition 06.11.02

Intended use
For in vitro diagnostic use.
DAKO Monoclonal Mouse Anti-Human CD20cy, B Cell, Clone L26, is intended for use in immunocytochemistry. The antibody labels cells of the B-cell lineage, and is a very useful tool for the identification of neoplasms of B-cell derivation (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.

Synonym for antigen
L26 (2).

Introduction
CD20 is a transmembrane, non-glycosylated protein expressed on B cell precursors and mature B cells, but is lost following differentiation into plasma cells (3). In resting B cells, CD20 appears in a 33 kDa non-phosphorylated form. After mitogen stimulation, CD20 becomes heavily phosphorylated (35-37 kDa isoforms), and it is a dominant phosphoprotein in activated B cells, B-cell lines, and hairy cell leukaemias (2). The long N- and C terminal ends of the protein are located on the cytoplasmic side of the membrane and only a minor portion of the protein is exposed on the cell surface (3). Antibodies reacting with CD20 cytoplasmic epitopes are designated CD20cy (2). It is suggested that CD20 plays a direct role in regulating the transmembrane conductive Ca²⁺ flux of B cells which indicates a possible function for CD20 as a regulator of proliferation and differentiation (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: L26 (1, 4). Isotype: IgG2a, kappa. Mouse Ig concentration: 384 mg/L. Total protein concentration: 24.5 g/L.

Immunogen
Human tonsil B cells (4).

Specificity
The antibody was clustered as anti-CD20 at the Fifth International Workshop and Conference on Human Leucocyte Differentiation Antigens held in Boston 1993 (2). SDS-PAGE analysis of immunoprecipitates formed between ¹²⁵I-labelled tonsil cell lysate and the antibody shows reaction primarily with 30 kDa and 33 kDa polypeptides (4). Studies using COS-1 cells transfected with cDNA encoding the CD20 molecule, indicate that the antibody labels an intracytoplasmic epitope localized on the CD20 molecule (5).

Precautions
1. For in vitro diagnostic use.
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.

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 on paraffin-embedded tissue sections fixed in formalin or B5 fixative (6). Pre-treatment of tissues with heat-induced epitope retrieval is recommended. For heat-induced epitope retrieval, the following solutions were found efficient: 10 mmol/L citrate buffer, pH 6.0; 10 mmol/L Tris buffer, 1 mmol/L EDTA pH 9.0; DAKO Target Retrieval Solution, High pH, code No. S 3008. Pre-treatment of tissues with proteinase K was found to destroy 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 may be used for analysis of acetone-fixed frozen sections (1) and acetone-fixed cell preparations (1, 4).
Staining procedure

**Dilution:** DAKO Monoclonal Mouse Anti-Human CD20cy, B Cell, code No. M 0755, may be used at a dilution range of 1:200-1:400 when applied on formalin-fixed, paraffin-embedded sections of human tonsil and using 15 minutes heat-induced epitope retrieval in 10 mmol/L citrate buffer, pH 6.0, 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. As a negative control, DAKO Mouse IgG2a, code No. X 0943, 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 smears, 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.

**Automation:** The antibody is well-suited for immunocytochemical staining using automated platforms, such as the DAKO Autostainer.

**Product-specific limitations**

Rare cases of CD20-positive peripheral T-cell lymphomas have been reported (8, 9).

**Performance characteristics**

**Normal tissues:** In normal lymphoid tissue, the antibody labelled germinal centre cells, mantle zone lymphocytes, and scattered interfollicular lymphocytes, but not T cells, histiocytes and plasma cells (6, 8). No labelling was observed in epidermis, sebaceous glands, hair follicles and eccrine glands in the skin, follicular epithelium in the thyroid, pneumocytes and bronchial epithelium of the lung, and a large number of other normal non-lymphoid tissues tested (6).

**Abnormal tissues:** Positive reaction with the antibody was revealed in most of 131 B-cell neoplasms tested (1). Labelling with the antibody shows that in the differentiation of B cells, the CD20 antigen is not expressed on very immature lymphoid cells (0/6 acute undifferentiated leukaemias), but begins to be expressed on early maturational stages (14/34 common acute lymphoblastic and 7/9 pre-B acute lymphoblastic leukaemias), and then, the CD20 antigen is fully expressed on mature B cells (15/15 chronic lymphocytic, 3/3 prolymphocytic, 3/3 hairy cell, 6/7 lymphosarcoma cell leukaemias, and 45/46 B-cell malignant lymphomas including Burkitt, Waldenström, and immunoblastic B-cell lymphomas. The CD20 antigen disappears on plasma cells, and only 1/2 plasma cell leukaemias and 0/12 myelomas were labelled by the antibody (1). Other studies have provided comparable results showing positive labelling of 44/44 large cell and immunoblastic B-cell lymphomas (6), and all of 40 B-cell lymphomas, with the exception of common acute lymphoblastic leukaemias and malignant lymphoma plasmacytic (8). In Hodgkin’s disease, strong surface membrane staining of Reed-Sternberg cells was observed in 9/27 cases (8).

Of lymphoproliferative diseases of T-cell lineage, 0/73 were labelled by the antibody (1), whereas other studies showed 1/18 (8) and 1/111 (9) positive cases.

**References**