Monoclonal Mouse
Anti-Human CD45, Leucocyte Common Antigen
Clones 2B11 + PD7/26
Code No. M 0701
Lot 101. Edition 05.02.03

Intended use
For in vitro diagnostic use.
Monoclonal Mouse Anti-Human CD45, Leucocyte Common Antigen, Clones 2B11 + PD7/26, is intended for use in immunocytochemistry. The antibody labels CD45 in both normal and neoplastic cells, and is a useful tool for identifying tumour cells of lymphoid origin (1-3). 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
T200, Ly-5.

Introduction
CD45 is a transmembrane glycoprotein expressed on most nucleated cells of haematopoietic origin. CD45, encoded by a single gene mapped to chromosome 1, has various isoforms based on differential splicing of exons 4, 5 and 6. On human leucocytes, five different isoforms of CD45, named ABC, AB, BC, B and 0, have been identified. These isoforms are recognized by CD45RA, CD45RB, CD45RC and CD45R0 antibodies. The isoforms range in Mr from 180 000 to 220 000. All the CD45 isoforms share the same intracellular segment, which has been shown to have tyrosine phosphatase activity. Various leucocytes express characteristic CD45 isoforms, thus T cells express CD45 isoforms corresponding to their development and activation, B cells predominantly express the ABC isoform, and monocytes and dendritic cells predominantly express the B and 0 isoforms. Granulocytes principally express only the B and 0 isoforms (4).

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: 2B11 (1) and PD7/26 (1). Isotype: IgG1, kappa. Mouse IgG concentration: 300 mg/L. Total protein concentration: 14.9 g/L.

Immunogen

Specificity
Anti-CD45 is a mixture of two monoclonal antibodies, clones 2B11 and PD7/26, directed against different epitopes. Clone 2B11 was clustered as anti-CD45 at the Third International Workshop and Conference on Human Leucocyte Differentiation Antigens, held in Oxford in 1986 and reacts with all the known isotypes of the CD45 family (5). Clone PD7/26 was clustered as anti-CD45RB at the Fifth International Workshop and Conference on Human Leucocyte Differentiation Antigens, held in Boston in 1993 (6).

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 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 our Technical Services.

Specimen preparation
Paraffin sections: The antibody can be used for labelling paraffin-embedded tissue sections fixed in formalin, B5 (2), or Bouin’s (1). Pre-treatment of tissues with heat-induced epitope retrieval is recommended. Optimal results are obtained with DakoCytomation Target Retrieval Solution, code No. S 1700, DakoCytomation Target Retrieval Solution, High pH, code No. S 3308, 10 mmol/L citrate buffer, pH 6.0, or 10 mmol/L Tris buffer, 1 mmol/L EDTA, pH 9.0. Pre-treatment of tissues with proteinase K was found destructive of 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 (1), and cell smears.

Staining procedure
Dilution: Monoclonal Mouse Anti-Human CD45, Leucocyte Common Antigen, code No. M 0701, may be used at a dilution range of 1:50-1:100 when applied on formalin-fixed, paraffin-embedded sections of human tonsil and using 20 minutes heat-induced epitope retrieval in DakoCytomation 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. The recommended negative control is DakoCytomation Mouse IgG1, code No. X 0931, diluted to the same mouse IgG concentration as the primary antibody.

"(2)"
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 DakoCytomation 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 DakoCytomation Autostainer.

Product-specific limitations

Labelling of the surface membrane of mammary ductal cells in specimens of fibrocystic disease and fibroadenoma has been reported for the antibody. This expression, however, is not considered to present a differential diagnostic problem that might lead to misidentification (7).

Performance characteristics

Cells labelled by the antibody predominantly display staining of the cell membrane, but cytoplasmic staining may also occur.

Normal tissues: In tonsil, the antibody labels germinal centres, follicular mantle zones, and interfolllicular regions (1). In spleen, white pulp and lymphoid cells of red pulp are positive, as also thymic lymphocytes, bone marrow lymphoid cells, mast cells, cells of probable monocytic derivation, and occasional plasma cells. Variable labelling of immunoblasts, epitheloid histiocytes, sinus histiocytes and plasma cells has been reported. Myeloid cells, erythroid cells, megakaryocytes, Langerhans cells in skin, epithelium, and connective tissue are not labelled by the antibody (2).

Abnormal tissues: In non-Hodgkin’s lymphoma, the neoplastic cells were labelled by the antibody in 40/40 (100%) of cases (1). In another study (2) the figure was 74/80 (93%). A third study (8) showed that 52/52 (100%) low grade B-cell lymphomas, 99/108 (92%) high grade B-cell lymphomas, and 41/44 (93%) T-cell lymphomas were positive with the antibody. Altogether 162/162 (100%) non-lymphoid neoplasms were negative with the antibody, including small cell anaplastic carcinomas, amelanotic melanomas, alveolar rhabdomyosarcomas, Ewing’s sarcoma, and germ cell tumours (1, 2).

References