Monoclonal Mouse Anti-Human Leukaemia, Hairy Cell
Clone DBA.44
Code No. M 0880
Lot 011. Edition 07.03.03

Intended use
For in vitro diagnostic use.

Monoclonal Mouse Anti-Human Leukaemia, Hairy Cell, Clone DBA.44, is intended for use in immunocytochemistry. The antibody labels more than 97.6% of hairy cell leukaemias (HCL) (1-3) and about 79% of splenic lymphomas with villous lymphocytes (SLVL) (4), and it is a useful tool for the identification of hairy cell leukaemia, particularly in the detection of minimal residual disease (5), and in the differentiation of HCL and SLVL from B-cell chronic lymphocytic leukaemia (2, 4). 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.

Reagent provided
Monoclonal mouse antibody supplied 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: DBA.44 (1). Isotype: IgM, kappa.
Mouse IgM concentration: 115 mg/L. Total protein concentration: 14.1 g/L.

Immunogen
DEAU cell line established from a diffuse large-cell lymphoma of centroblastic type (1).

Specificity
The DBA.44 antibody recognizes an unknown, fixation-resistant antigen expressed by mantle zone lymphocytes, reactive immunoblasts, monocytoïd B cells, and a small proportion of high- and low-grade lymphomas (1, 2).

Western blotting have been unsuccessful in determining the molecular mass of the antigen recognized by the antibody (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, 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 our Technical Services.

Specimen preparation
Paraffin sections: The antibody can be used for labelling paraffin-embedded tissue sections fixed in formalin, B5, Bouin’s, or Bouin’s derivative (1, 2). Pre-treatment of tissues with heat-induced epitope retrieval is required. For tissues fixed in formalin, 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 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 (1), and acetone-fixed cell preparations (3).

Staining procedure
Dilution: Monoclonal Mouse Anti-Human Leukaemia, Hairy Cell, code No. M 0880, may be used at a dilution range of 1:25-1:50 when applied on formalin-fixed, paraffin-embedded sections of human tonsil or sections of bone marrow or spleen from a patient with hairy cell leukaemia and using 20 minutes heat-induced epitope retrieval in DakoCytomation Target Retrieval Solution, code No. S 1700, DakoCytomation Target Retrieval Solution, High pH, code No. S 3308, 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 IgM, code No. X 0942, diluted to the same mouse IgM concentration as the primary antibody.

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.

Product-specific limitations
In normal non-lymphoid tissues, the antibody shows cross-reactivity with lung alveolar lining cells, kidney tubules, some endothelial cells and salivary duct cells (1).
Performance characteristics

Cells labelled by the antibody display a staining confined to the cell membrane, but a dot-like paranuclear reaction is observed in immunoblasts (1, 2).

Normal tissues: In lymph nodes the antibody labelled endothelial cells, the cytoplasm of a few macrophages which had phagocytized antigen, and, in some cases, a few germinal centre cells. In thymus, only scarce medullary lymphocytes were labelled. In reactive lymph nodes and spleen, the antibody labelled cytoplasmic membranes of the mantle zone cells and some immunoblasts outside lymphoid follicles. In non-lymphoid tissues the antibody labelled lung alveolar cells, kidney tubules, some endothelial cells, and salivary duct cells (1).

Abnormal tissues: Of hairy cell leukaemias, 41/42, 238/241, and 41/41 showed a strong positive labelling of surface membrane hairy features with the antibody (1-3). Of splenic lymphomas with villous lymphocytes, 19/24 were labelled by the antibody (4). HCL and SLVL could be distinguished by their cytological features (4). Among B-cell chronic lymphocytic leukaemias, 0/8, 2/42, and 1/21 were positive (1, 2, 4).

In a range of T-cell lymphomas, 3/83 (3.6%) were positive with the antibody, but the staining was restricted to the paranuclear area in only a few large cells (2). In a panel representing 25 different non-lymphoid tumours, the antibody demonstrated weak staining of a small number of cells in 1/4 carcinoid tumours and 1/2 adenocarcinomas of colon and rectum (1).

References