**Monoclonal Mouse Anti-Human CD30**  
**Clone Ber-H2**  
**Code No. M 0751**  
**Lot 051. Edition 29.11.01**

**Intended use**  
For in vitro diagnostic use.

DAKO Monoclonal Mouse Anti-Human CD30, Clone Ber-H2, is intended for use in immunocytochemistry. The antibody labels anaplastic large-cell lymphoma (ALCL) and Reed-Sternberg cells, and is a useful tool for the identification of ALCL and as a secondary marker for Hodgkin’s disease (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**  
Ki-1 antigen (1).

**Introduction**  
CD30 is a transmembrane cytokine receptor belonging to the tumour necrosis factor (TNF) receptor superfamily. Mature CD30 has a molecular mass of 120 kDa and is derived from a 90 kDa precursor protein (2). The extracellular domain of CD30 is homologous to that of TNF receptor superfamily members, whereas there is no homology in the cytoplasmic domain, suggesting major differences in signalling mechanisms (3). The intracellular part of CD30 possesses kinase activity, indicating that CD30 plays a role in regulating the function, differentiation and/or proliferation of normal lymphoid cells (2). A soluble 85 kDa form of CD30, sCD30, released from the membrane-bound molecule by proteolytic cleavage, can be detected in the sera of patients with CD30-expressing neoplasms (3, 4).

CD30 expression is found on Hodgkin and Reed-Sternberg (H-RS) cells, anaplastic large-cell lymphoma cells, and on activated B and T lymphocytes (2). In non-lymphoid tissues and neoplasms, CD30 expression has been confirmed in embryonal carcinomas, seminomas, decidual cells and mesotheliomas (5).

**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: Ber-H2 (1). Isotype: IgG1, kappa.

Mouse IgG concentration: 350 mg/L. Total protein concentration: 22.7 g/L.

**Immunogen**  
Co cell line established from a patient with Hodgkin’s disease of T-cell lineage (1, 6).

**Specificity**  
The antibody was clustered as anti-CD30 at the Fourth International Workshop and Conference on Human Leucocyte Differentiation Antigens held in Vienna in 1989 (7).

SDS-PAGE analysis of immunoprecipitates formed between lysate of ¹²⁵I-labelled COS cells transfected with cDNA encoding CD30 and the antibody shows reaction with a 120 kDa protein corresponding to CD30. Mock-transfected COS cells were negative. The epitope recognized by the antibody is located between amino acid residues 112 and 412 (8).

The antibody labels: Cell lines derived from Hodgkin’s disease, L428, L540, L591, Co, Ho and KM-H2; HTLV-1 transfected T-cell lines, Hut-102 and MT-2; EBV-transformed B-cell lines (non-Burkitt), B95-8 (monkey), BJA-B and Cess; and the myeloid cell line, K 562 (1).

**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 on paraffin-embedded tissue sections fixed in formalin. Heat-induced epitope retrieval in 10 mmol/L citrate buffer, pH 6.0 (5) is recommended. Proteolytic pretreatment of tissues was found less optimal (5). 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 and cytopsin preparations (1, 5).
Staining procedure

**Dilution**: DAKO Monoclonal Mouse Anti-Human CD30, code No. M 0751, may be used at a dilution range of 1:20-1:40 when applied on formalin-fixed, paraffin-embedded sections of Hodgkin's lymphomas or ALCCL 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 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, 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

A diffuse or finely granular cytoplasmic staining was observed in the endothelial cells in 21/33 cases of haemangiomia, 4/10 cases of lymphangiomia, 4/9 cases of mixed tumours with both components, and 6/10 cases of angioleiomyoma (9).

Performance characteristics

Cells labelled by the antibody display a membrane and/or a dot like cytoplasmic staining (1).

**Normal tissues**: In tonsil/lymph node sections, the antibody labels scattered large lymphoid cells localized around lymph follicles and at the margin of germinal centres. In paraffin sections, but not in frozen sections, a subpopulation of plasma cells is positive. In the thymus, only a few medullary thymocytes is labelled. In a large range of non-lymphoid tissues, no labelling with the antibody was observed with two exceptions, the cytoplasm of exocrine pancreatic cells was labelled diffusely in both frozen and paraffin-embedded sections, and the cytoplasm of a proportion of cerebral cortical neurons and Purkinje cells of the cerebellum were labelled in paraffin-embedded sections, but not frozen sections. The antibody did not react with any resting peripheral lymphocytes or monocytes (1).

**Abnormal tissues**: In paraffin-embedded sections of anaplastic large-cell lymphoma, the antibody strongly labelled all of 60 cases. No differences were observed in labelling between T-, B- or null-cell phenotypes. Likewise, all of 22 frozen sections of Hodgkin’s disease, 61/61 cases of nodular sclerosis, 53/53 cases of mixed cellularity, 8/10 cases of lymphocytedepleted, and 4/13 cases of lymphocyte-predominance type were labelled by the antibody. In frozen sections, all of 108 tested cases were positive (1). In non-Hodgkin’s lymphomas, a weak staining of a subpopulation of tumour cells was seen in 11/11 cases of lymphomatoid papulosis, 62/93 cases of cutaneous-, pleomorphic-, angioimmunoblastic-, and lymphoepitheliod- T-cell lymphomas, and in 53/332 cases of chronic lymphocytic-, centrocytic- (small cleaved), centroblastic-centrocytic-, centroblastic-, and immunoblastic- B-cell lymphomas in frozen and paraffin-embedded sections, whereas a strong staining was seen in 20/67 cases of lymphoplasmocytoid/cytic-B-cell lymphomas (1). In non-lymphoid neoplasms, the antibody labels tumour cells in 48/50 cases of pure embryonal carcinoma (EC), or EC components of germ cell tumours. In cases of mixed and pure germ cell tumours without EC components 0/27 was labelled. In activated mesothelium, 16/28 pleural and peritoneal effusions were positive with the antibody, small foci of tumour cells in 2/8 mesotheliomas were also positive (5). No labelling was observed in 8 cases of Kaposi’s sarcoma and 8 cases of teleangiectatic granuloma (9).

References