Monoclonal Mouse Anti-Human Cathepsin D
Clone DB2000
Code No. M 7243
Lot 051. Edition 19.07.01

Intended use
For in vitro diagnostic use.
DAKO Monoclonal Mouse Anti-Human Cathepsin D, Clone DB2000, is intended for use in immunocytochemistry. The antibody labels the cathepsin D molecule in normal and neoplastic tissues and may be a useful tool in the assessment of overexpression of cathepsin D in cancer, which is thought to be associated with tumour cell proliferation and spread. Interpretation must be made within the context of the patient’s clinical history and other diagnostic tests by a qualified pathologist.

Introduction
Cathepsin D is an aspartic protease expressed in all mammalian tissues with the exception of erythrocytes. It is found in endosomes and lysosomes and comprises approximately 10% of the total protein in lysosomes (1, 2). Human cathepsin D is synthesized as a pro-enzyme, and is processed sequentially into the mature two-chain form comprising a 14 kDa light chain and a 34 kDa heavy chain. Cathepsin D is involved in protein degradation, and has also been suggested as an activator of precursors of biologically active molecules (3).

In breast cancer, a strong positive association was found between overexpression of cathepsin D and the presence of tumour in axillary lymph nodes (4) or, generally, metastatic disease (5). Also in laryngial carcinoma (6), colon adenocarcinoma (7), bladder carcinoma (8), prostate carcinoma (9), and glioma (10) was cathepsin D overexpression positively correlated to pathologic stage.

Reagent provided
Monoclonal mouse antibody provided in liquid form as mouse IgG purified from cell culture supernatant. In 0.05 mol/L Tris/HCl, 15 mmol/L NaN₃, 1% bovine serum albumin, pH 7.2. Package size is 1 mL.
Mouse IgG concentration: 44 mg/L. Total protein concentration: 10 g/L.

Immunogen
Cathepsin D isolated from human liver (3).

Specificity
In Western blots of isolated, reduced human cathepsin D, the antibody labels a band of 34 kDa, corresponding to the heavy chain of mature cathepsin D (3).
In ELISA the antibody recognizes cathepsin D, but none of the other tested lysosomal cathepsins (B, H, L, S) (3).

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. Heat-induced epitope retrieval in: 10 mmol/L citrate buffer, pH 6.0; 10 mmol/L Tris buffer, 1 mmol/L EDTA, pH 9.0; DAKO Target Retrieval Solution, code No. S 1700; or DAKO Target Retrieval Solution, High pH, code No. S 3308, is required. Pre-treatment of tissues with proteinase K was found inefficient for epitope retrieval. The tissue sections should not dry out during the treatment or during the following immunocytochemical staining procedure.

Staining procedure
Dilution: DAKO Monoclonal Mouse Anti-Human Cathepsin D, code No. M 7243, may be used at a dilution range of 1:25-1:50 when applied on formalin-fixed, paraffin-embedded sections of tonsil or breast carcinoma and using 15 minutes heat-induced epitope retrieval in 10 mmol/L citrate buffer, pH 6, 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 kits, code Nos. K 0679 and K 0690, and DAKO EnVision®+/ HRP kits, code Nos. K 4006 and K 4007, 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.

Performance characteristics

Cells labelled by the antibody display a strong granular staining in the cytoplasm.

References


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