Monoclonal Mouse Anti-Human CD68, Macrophage
Clone PG-M1
Code No. M 0876
Lot 022, Edition 14.03.02

Intended use
For in vitro diagnostic use.
DAKO Monoclonal Mouse Anti-Human CD68, Macrophage, Clone PG-M1, is intended for use in immunocytochemistry. The antibody labels macrophages and is a useful tool for the identification of M4 (myelomonocytic) and M5 (monocytic) types of acute myeloid leukaemia (AML), and histiocytic sarcoma (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.

Introduction
CD68 is a highly glycosylated lysosomal membrane protein with an Mr of 110,000. The CD68 protein belongs to a family of lysosomal glycoprotein (LGP)/plasma membrane shuttling proteins that play a role in endocytosis and/or lysosomal trafficking.
CD68 is expressed strongly in cytoplasmic granules, and weakly on the surface of macrophages, monocytes, neutrophils, basophils and NK-cells. Additionally, CD68 is expressed by approximately 40% of peripheral blood B cells and is weakly expressed in 50% of B-cell type acute lymphoblastic leukaemia (B-ALL) cells. CD68 can also be found in the cytoplasm of non-haematopoietic tissues, especially the liver, and renal glomeruli and tubules (2). Unlike many other CD leucocyte antigens, the CD68 molecule is antigenically very heterogeneous, and different antibodies to CD68 show different cellular reactivities (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.
Mouse IgG concentration: 190 mg/L. Total protein concentration: 8.6 g/L.

Immunogen
Human mononuclear spleen cell preparation containing more than 80% Gaucher's cells (3).

Specificity
The antibody was clustered as anti-CD68 at the Fifth International Workshop and Conference on Human Leucocyte Differentiation Antigens held in Boston in 1993 (4).
The antibody labels COS-1 and WOP cells transfected with CD68 cDNA. Unlike other CD68 antibodies, which label both macrophages and myeloid cells, the PG-M1 antibody detects a fixative-resistant epitope on the macrophage-restricted form of the CD68 antigen (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, Bouin's or B5 (3). Epitope retrieval is required, either 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; or proteolytic pre-treatment with chymotrypsin, trypsin, pepsin, pronase (3) or proteinase K. 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, although the labelling is slightly weaker than that observed in paraffin sections (3, 4). It is also well-suited for cell preparations (3).

Staining procedure
Dilution: DAKO Monoclonal Mouse Anti-Human CD68, Macrophage, code No. M 0876, 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 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.
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 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 The PG-M1 antibody labels some non-haematopoietic malignancies, especially about 10% of melanomas (3), therefore the diagnosis of histiocytic sarcoma should always be supported by positivity of neoplastic cells for at least another macrophage-restricted marker (for example using DAKO code No. M 0794, clone Ber-MAC3) and/or the leucocyte common antigen, and negativity for epithelial and melanoma-associated antigens (1).

Performance characteristics Cells of the monocyte/macrophage lineage labelled by the antibody show a cytoplasmic (diffuse or granular) staining pattern.

Normal tissues: In normal peripheral blood only monocytes are labelled by the antibody. In a wide range of tissues tested, all macrophages were labelled. In bone marrow also osteoclasts were positive, whereas granulocytes and myeloid precursors were consistently negative. A weak reactivity of some megakaryocytes was observed in about 20% of the cases. Kupffer's cells in the liver, mast cells, and synovial cells were the only additional normal cells labelled by the antibody (3).

Abnormal tissues: Among 431 malignancies of the lymphohemopoietic system, reactivity with the antibody was restricted to acute myeloid leukaemias of the M4 and M5 type, true histiocytic sarcomas, and mastocytosis. Consistently negative were acute myeloblastic leukaemias of M1, M2, and M3 type, malignant non-Hodgkin's lymphomas of B- and T-cell type, Hodgkin's lymphoma, acute lymphoblastic leukaemias, and chronic myeloid leukaemia. The majority of 370 non-haematopoietic tumours were negative with the antibody, exceptions being 15/15 granular cell myoblastomas, 6/13 kidney clear cell carcinomas, 4/10 glioblastomas, 10/18 meningiomas and 5/50 melanomas, and, as expected, 2/2 giant cell tumours of the bone, and 7/7 xanthogranulomas (3).

References


