Monoclonal Mouse Anti-Human Granzyme B
Clone GrB-7
Code No. M 7235
Lot 0002563. Edition 15.07.03

Intended use
For in vitro diagnostic use. Monoclonal Mouse Anti-Human Granzyme B, clone GrB-7, is intended for use in immunocytochemistry. The antibody labels activated human cytotoxic T lymphocytes (CTL) and natural killer (NK) cells, and is a useful tool for the identification of the neoplastic counterparts of these cells (1). Additionally, combined granzyme B, perforin, and Fas ligand immunocytochemical labelling has been reported to increase accuracy of renal allograft rejection diagnosis (2). 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
Granzyme B, a 29 kDa monomer protein, is a member of a subfamily of serine proteases with chymotrypsin-like specificity. For CTL and NK cells to exert their cytolitic activity, the presence of cytoplasmic secretory granules containing granzymes and perforin are pivotal. After granule exocytosis, granzyme B is believed to enter the target cell through a perforin-derived transmembrane channel to induce DNA fragmentation and apoptosis. A high percentage of activated CTL’s, identified by anti-granzyme B, clone GrB-7, and present in tumour biopsies of patients with nodular sclerosis or mixed cellularity Hodgkin’s disease, has been reported to be a strong indicator for an unfavorable outcome (3).

Reagent provided
Monoclonal mouse antibody provided in liquid form as purified IgG from cell culture supernatant. In 0.05 mol/L Tris/HCl, 15 mmol/L NaN3, 1% bovine serum albumin, pH 7.2.
Clone: GrB-7 (4, 5). Isotype: IgG2a, kappa.
Mouse IgG concentration: 70 mg/L. Total protein concentration: 10 g/L.

Immunogen
Recombinant human granzyme B (4, 5).

Specificity
In Western blotting of IL-2 stimulated PBMNC lysates, the antibody labels a band corresponding to granzyme B (4, 5).
In immunocytochemistry, the antibody labels activated CTL and NK cells (4, 5).

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. Pre-treatment of tissues with heat-induced epitope retrieval is required. Optimal results are obtained with DakoCytomation Target Retrieval Solution, High pH, code No. S 3308, or 10 mmol/L Tris buffer, 1 mmol/L EDTA, pH 9.0. The following solutions DakoCytomation Target Retrieval Solution, code No. S 1700 and 10 mmol/L citrate buffer, pH 6.0 or pre-treatment of tissues with Proteinase K, code No. 3020, was found inefficient. The tissue sections should not dry out during the treatment or during the following immunocytochemical staining procedure.
Frozen sections and cell preparations: The antibody cannot be used for labelling frozen sections (5).

Staining procedure
Dilution: Monoclonal Mouse Anti-Human Granzyme B, code No. M 7235, may be used at a dilution range of 1:25-1:50 when applied on formalin-fixed, paraffin-embedded sections of human tonsil or CTL preparations and using 20 minutes heat-induced epitope retrieval in 10 mmol/L Tris buffer, 1 mmol/L EDTA, pH 9.0, 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. The recommended negative control is DakoCytomation Mouse IgG2a, code No. X 0943, diluted to the same mouse IgG 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. Follow the procedure enclosed with the selected visualization kit.
Product-specific limitations

Used alone, Anti-Granzyme B may not reliably give a differential diagnosis of tumour origin. Rather, it should be applied in conjunction with antibodies against other cytotoxic granula markers, such as perforin and TIA-1.

Performance characteristics

Cells labelled by the antibody display a diffuse cytoplasmic staining pattern corresponding to the granular localization of the antigen.

Normal tissues: The antibody labels a high number of cells in the red pulp of the spleen. Significant numbers of positive cells are found in tonsil, lymph nodes, liver and thymus. Low numbers are present in the lamina propria of non-inflamed stomach, small intestine and colon (5).

Abnormal tissues: In reactive lymph nodes, the antibody strongly labels a subset of paracortical lymphocytes. In 5 peripheral T cell lymphomas with cytotoxic phenotype (3 intestinal T cell lymphomas (ITL), 1 anaplastic lymphoma (ALCL), and 1 nodal peripheral T cell lymphoma (PTL)), antibody labelling was observed in 2/3 ITL, 1/1 PTL and 1/1 ALCL. 19/19 T/NK-cell lymphomas were positive with the GrB-7 antibody (1). Activated CTLs in patients with Hodgkin’s disease were also labelled by this antibody (3).

References