Rabbit Anti-Human
CD3, T Cell
Code No. A 0452
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Intended use
For in vitro diagnostic use.

DAKO Rabbit Anti-Human CD3, T cell is for use in immunocytochemistry. It is intended for laboratory use to semi-quantitatively identify by light microscopy the CD3 molecule in normal and pathological human paraffin-embedded specimens processed in neutral-buffered formalin. Positive results aid in the classification of normal and abnormal cells/tissues and serve as an adjunct to conventional histopathology. The clinical interpretation should be complemented by morphological and histological studies. Used alone, the antibody may not reliably give a differential diagnosis of tumour origin. Rather, it should be applied in conjunction with antibodies against other T cell-associated markers. Evaluations must be made within the context of the patient’s clinical history and other diagnostic tests by a qualified pathologist.

The antibody is of value to histopathologists since it labels T cells in routinely fixed, paraffin-embedded tissue. The antibody can thus be used to immunophenotype T cell neoplasms and reactive lymphocytes when no fresh material is available (1).

Synonym for antigen
Leu-4, T3 antigen.

Introduction
The CD3 molecule consists of five polypeptide chains; gamma (γ), delta (δ), epsilon (ε), zeta (ζ) and eta (ϕ) with molecular masses ranging from 16-28 kDa. The molecule was designated CD3 at the First International Workshop on Human Leukocyte Differentiation Antigens in Paris 1982 (2).

CD3 is first detectable in early thymocytes and its appearance probably represents one of the earliest signs of commitment to the T cell lineage (3). In cortical thymocytes the antigen is predominantly present as an intracytoplasmic constituent. It appears subsequently, at the medullar thymocyte stage, on the T cell surface in close association with the T cell receptor (TcR). Whereas the TcR forms the ligand-binding part of the TcR/CD3 complex, the function of CD3 molecule is that of signaling, making CD3 a highly specific marker for T cells. No other cells are known to express the CD3 molecule, although two monoclonal antibodies raised against CD3 has been found to react with Purkinje cells in the cerebellum (4).

The CD3 molecule is present in the great majority of T cell neoplasms, although occasional tumours are encountered in which the antigen is lost as part of the neoplastic process (5). The CD3 molecule is expressed in some cases of malignant histiocytosis (6) and in Hodgkin's disease (7).

The CD3ε and not the whole CD3 molecule has been detected in natural killer (NK) cells (8), and the expression of CD3ε may be a marker of nasal T cell lymphomas which is thought to be of NK cell origin (9).

Reagent provided
The antibody is an affinity-isolated antibody. The affinity isolation has been performed using immobilized CD3 peptide.

Total protein concentration: 0.5 g/L.

Immunogen
A synthetic peptide, corresponding to amino acids 156-168 from the cytoplasmic domain of the human CD3ε (10), to which a terminal cysteine was added, was coupled to thyroglobulin and used for immunization (1).

Specificity
In immunocytochemistry the antibody shows specific staining of human T cells in both the cortex and medulla of the thymus and in peripheral lymphoid tissues (1). The antibody cross-reacts with CD3 in mouse (11) and koala (12).

In immunoprecipitation from Nonidet P40 lysates of surface-iodated T lymphoblasts, the antibody has shown to precipitate the γ (26 kDa), δ (21 kDa) and ε (19 kDa) chain of the CD3 molecule similar to the precipitation pattern seen using the well-characterized Monoclonal Mouse Anti-Human CD3, Clone UCHT1 (1).

In Western blotting the antibody detects bands of the expected molecular weights for CD3 antigens (13). The antibody recognizes the CD3ε in both T cells and NK cells, but do not react with lysates prepared from B cells, myeloid or colon carcinoma cells (8). The antibody may also be used for identification of koala CD3 in Western blotting (12).

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 azides. 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, which has been fixed in formalin or in Bouin's fixative (14), whereas fixation in B5 (13) may result in suboptimal staining. Heat-induced epitope retrieval by boiling in 10 mmol/L citrate buffer, pH 6.0 (16) or in DAKO Target Retrieval Solution for 15 minutes is recommended. Epitope retrieval such as trypsin (1) and protease (14, 15) have been tested but is suboptimal. The slides should not be allowed to dry out during this treatment or during the following immunocytochemical staining procedure.

Frozen sections and cell smears: May be used for analysis of cryostat sections (1, 9, 12, 15).

Staining procedure

Dilution: The antibody may be diluted 1:50-1:100 when using citrate buffer, pH 6.0, for epitope retrieval with the LSAB®+ or the EnVision™+ systems and tested on formalin-fixed, paraffin-embedded sections of human tonsil. The optimal dilution may vary depending on specimen and preparation method, and should be determined by each individual laboratory. The recommended incubation time for this primary antibody is 30 minutes at room temperature (18-25°C).

Visualization: Sensitive techniques such as the LSAB®+ or the EnVision™+ systems are recommended. However, the APAAP technique may be used (1).

Automation: The antibody is suitable for immunocytochemical staining using automated platforms, such as the DAKO Autostainer.

Product-specific limitations

Not known.

Performance characteristics

The antibody stains both the cytoplasm and the membrane of CD3ε-positive T cells (7). When staining fetal and adult NK cells only the cytoplasm is stained (8). The antibody reacts with both normal and neoplastic tissue of T cell (1, 15) and NK cell origin (8, 9).

References