DAKO Dual Colour Reagent
Anti-CD3/FITC, clone UCHT1 +
Anti-CD16/RPE, clone DJ130c
Code No. FR 770
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Introduction
Human TCR/CD3 is a complex structure on the lymphocyte surface. It consists of the clonotypic TCR heterodimers, αβ or γδ. The CD3 part comprises at least four invariant types of chains (γ, δ, ε, ζ) (1). Stoichiometry and other data propose a monovalent TCRαβCD3γεδεζζ octamer, but a bivalent TCR in a decameric complex has also been suggested (2). Most CD3 antibodies are directed against the 20 kDa ε-chain (3).

CD3 is closely associated with the T cell antigen receptor TCR on the lymphocyte cell surface (4-6). It is believed that the CD3 complex homodimer (1, 4, 5) transduces activational signals to the interior of the cell upon antigen recognition by TCR. CD45, a protein tyrosine phosphatase, is a potent regulator of signal transduction by the CD3 complex (7).

The CD3 antigen is first detectable in early thymocytes and its appearance probably represents one of the earliest signs of commitment to the T cell lineage (8). In cortical thymocytes the antigen is predominantly present as an intracytoplasmic constituent (9). It appears subsequently (at the medullary thymocyte stage) on the T cell surface (8, 10).

The CD16 antigen is a 45-75 kDa glycoprotein identical to the low-affinity Fc receptor for complexed IgG (FcγRIII) that is expressed on natural killer (NK) cells, neutrophils and basophils. The CD16 exists both as a glycosyl-phosphatidyl (GPI)-anchored protein (FcγRIIIaM) in polymorphonuclear cells (PMN) and as a transmembrane-anchored protein (FcγRIIIbM) in NK cells (11-15).

The CD16 molecule represents the functional receptor in antibody-dependent cellular cytotoxicity (ADCC) (16).

Presentation
This dual colour reagent for flow cytometry is a combination of the following, carefully matched, fluororescent antibodies.
Anti-CD3, UCHT1 (9), a purified monoclonal mouse antibody conjugated with fluorescein isothiocyanate isomer 1.
Anti-CD16, DJ130c, a purified monoclonal mouse antibody conjugated with R-phycoerythrin (RPE).
Solvent: 0.05 mol/L Tris/HCl, 0.1 mol/L NaCl, 15 mmol/L NaN3, pH 7.2, 1% bovine serum albumin.
Isotype: IgG1, kappa.
Amount per vial: 50 tests (10 µL of reagent to 10^5 normal human peripheral blood mononuclear cells).

Storage
In the dark at 2-8 °C.

Specificities/reactivities
Anti-CD3, UCHT1, was included in the First, Third and Fourth International Workshops and Conferences on Human Leucocyte Differentiation Antigens (Paris 1982, Oxford 1986, Vienna 1989), and studies by a number of laboratories confirmed its reactivity with the CD3 antigen (17). Anti-CD3, UCHT1, reacts with the 20 kDa ε-chain of CD3 (3).

The antibody reacts with T cells in thymus, bone marrow, peripheral lymphoid tissue and blood (8, 10, 18, 19). The majority of T cell neoplasms also express the CD3 antigen, but it is absent from non-T cell lymphoid malignancies (20). Consistent with the pattern of synthesis of the antigen in normal thymocytes, the earliest site detectable within neoplastic cells is the cell cytoplasm (8).

This antibody is also able to induce in vitro proliferation of mature thymocytes and T cells in the presence of interleukin-2 (IL-2) (21).

Anti-CD16, DJ130c, was included in the Fifth International Workshop and Conference on Human Leucocyte Differentiation Antigens (Boston 1993), and studies by a number of laboratories confirmed its reactivity with the CD16 antigen (22). It reacts with NK cells, neutrophils and basrophils in peripheral blood and bone marrow.

The specificity of the antibody is equivalent to Leu-11a (clone NKP15).

Applications
DAKO Dual Colour Reagent, code No. FR 770, has been developed especially for use in flow cytometry. It allows simultaneous detection and enumeration of T cells and natural killer cells (23).

Staining procedure
1. Collect venous blood into a tube containing an anticoagulant.
2. Transfer 100 µL of the anticoagulated blood into a test tube.
3. Add 10 µL of FR 770. Mix gently. The reagent might be used neat or at a dilution of up to 1:10. This is a guideline only; the optimal dilution should be determined by the individual laboratory.
4. Use a non-reactive FITC and RPE-conjugated reagent of the same isotype, e.g. X 0932, as a negative control.
5. Incubate in the dark for 30 minutes at 4 °C.
6. Add 1-2 mL erythrocyte lysing reagent of your choice to each tube and mix gently. Follow the reagent manufacturer's recommendations for time and temperature of incubation.
7. Centrifuge at 300 x g for 5 minutes.
8. Aspirate the supernatant, leaving approximately 50 µL of fluid.
9. Add 3 mL 0.01 mol/L PBS containing 2% bovine serum albumin. Vortex gently.
10. Centrifuge at 300 x g for 5 minutes, then aspirate the supernatant, leaving approximately 50 µL of fluid.
11. Resuspend pellet in an appropriate fluid for flow cytometry analysis, e.g. 0.3 mL 1% paraformaldehyde (fixative) in PBS.
12. Analyse on a flow cytometer.

References