

OptiClone®

Monoclonal Antibodies
CD3 FITC / CD19 PE / CD45 PE-Cy5

50 Tests Cat. No. 1671

For research use only

Not for use in diagnostic procedures.

1. INTENDED USE

In this three-in-one OptiClone Monoclonal Antibody combination, the CD45 PE-Cy5 component is intended for flow cytometric identification of the lymphocytes within the leucocytes and their discrimination from debris, using an analysis of side scatter versus CD45 PE-Cy5 fluorescence (1,2). Then the CD3 FITC / CD19 PE combination is intended for simultaneous analysis of CD3⁺ T cells and/or CD19⁺ B cells.

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2. PRINCIPLE OF THE TEST

The monoclonal antibodies UCHT-1 and J4.119 bind to surface antigens present on human lymphocytes. UCHT-1 has been classified as CD3 and J4.119 as CD19 based on standard nomenclature (3,4). The monoclonal antibody Immu19.2 binds to the CD45 antigen isoforms which are expressed on the surface of human leucocytes (5).

CD3 (UCHT-1) is conjugated to fluorescein isothiocyanate (FITC); CD19 (J4.119), to R-phycoerythrin (PE); and CD45 (Immu19.2), to a tandem dye (PE-Cy5) which consists of R-phycoerythrin (PE) covalently coupled to cyanine 5 (Cy5). Upon excitation at 488 nm, each fluorescent dye emits light at different wavelengths, making possible the simultaneous study of CD3, CD19 and CD45 antigens.

This CD3 / CD19 / CD45 formulation is optimized for flow cytometric use.

After the whole blood sample is incubated with the fluorescent antibody combination, the red blood cells are lysed, using a no-wash procedure. The processed sample is then analyzed by flow cytometry.

The use of a side scatter versus CD45 PE-Cy5 fluorescence representation permits discrimination of lymphocytes (low side scatter level and bright CD45 staining) from unlysed red blood cells, red blood cell ghosts, platelet aggregates, and/or other debris (all CD45-negative events) (1-2). A gate may be drawn around the lymphocytes using CD45 PE-Cy5 fluorescence and side scatter parameters only. The events in the gate have been shown to be lymphocytes (1-2).

Using the side scatter versus CD45 PE-Cy5 histogram to define the lymphocyte gate, a fluorescent dual color representation of CD19 PE versus CD3 FITC can then be collected and the percentages of CD3⁺ or CD19⁺ populations be precisely determined.

3. REAGENT AND MATERIALS

Reagent provided: one vial containing 1 mL (50 tests) of OptiClone CD3 FITC / CD19 PE / CD45 PE-Cy5 Monoclonal antibodies.

CD3: Clone UCHT-1 was produced by the fusion of spleen cells from Balb/c mice immunized with human peripheral blood lymphocytes with the murine NS1 myeloma cell line. This monoclonal antibody is thought to recognize the ϵ chain of CD3 (6,7).

CD19: Clone J4.119 was produced by the fusion of spleen cells from Balb/c mice immunized with the human SKLY 18 lymphoma cell line with the mouse NS1 myeloma cell line.

CD45: Clone Immu19.2 was produced by the fusion of spleen cells from Balb/c mouse immunized with Epstein-Barr virus (EBV)-transfected human B-cells and the murine X63Ag8.653 myeloma cell line. This monoclonal antibody is thought to recognize all isoforms of CD45 molecules expressed on human leucocytes. **Immunoglobulin (Ig) Structure:** The three antibodies are comprised of mouse IgG1 heavy chains and kappa light chains.

Buffer: Phosphate-buffered saline (PBS: 0.01 M sodium phosphate, 0.145 M sodium chloride, pH 7.2) with 2.0 mg/ml bovine serum albumin (BSA).

Preservative: 0.1% (w/v) sodium azide.

Conjugation: CD3 FITC; conjugated with fluorescein isothiocyanate (3-6 moles FITC / mole Ig). The excitation wavelength peak of FITC is near 488 nm (blue-green); its emission peak is near 525 nm (green). CD19 PE; conjugated with R-phycoerythrin at 1 mole PE / mole Ig. R-phycoerythrin is excited at 488 nm, with its emission peak near 575 nm (orange-red). The CD45 antibody is conjugated with R-phycoerythrin-cyanine 5 (PE-Cy5) at a molar ratio of about 1 PE-Cy5 per Ig. Upon excitation at 488 nm the PE-Cy5 emits near 670 nm (deep-red).

WARNING

This reagent contains sodium azide as a preservative. Under acidic conditions sodium azide yields hydrazoic acid, an extremely toxic compound. Azide compounds should be diluted with running water before disposal to avoid deposits in plumbing where explosive conditions may develop.

If skin or eye contact occurs, wash excessively with water.

4. REAGENTS AND MATERIALS REQUIRED BUT NOT PROVIDED

1. OptiClone IgG1 FITC / IgG1 PE / CD45 PE-Cy5 Negative Control Monoclonal Antibodies (Cat. No 1673).
2. OptiLyse® Lysing Solution (Cat. No 1400 or 1401)
3. Phosphate-buffered saline (0.01 M sodium phosphate, 0.145 M sodium chloride).
4. Evacuated blood collection tubes with anticoagulant (EDTA, ACD, Heparin)
5. Polystyrene tubes (12 x 75 mm).
6. Pipettors and pipet tips capable of delivering 20 μ L and 100 μ L.
7. Vortex mixer.
8. Flow cytometer.
9. Blood cell counter.

5. STATEMENT OF WARNING

For research use only. Not for use in diagnostic procedures.

WARNING

Blood samples should be handled as if infectious. The biological samples and anything used to process them, should be disposed of by proper biohazard procedures.

6. REAGENT PREPARATION

OptiClone Monoclonal Antibodies are optimized for flow cytometric use and should be used directly from the vial with no further dilution or preparation. Do not expose to direct light for prolonged periods.

7. REAGENT STORAGE

OptiClone Monoclonal Antibodies are stable until the expiration date printed on each vial if stored at 2-8°C and protected from freezing and light.

B. BLOOD SAMPLE COLLECTION

Peripheral blood should be obtained by aseptic venipuncture. A salt of EDTA (K3 EDTA) is recommended. However, ACD and heparin may also be used (8). All blood specimens should be treated as if potentially infectious.

Store anticoagulated whole blood at 18-25°C. Specimens should be processed within 6 hours when possible. If not possible, the laboratory should verify that holding time and conditions maintain specimen integrity comparable to the fresh specimens.

Do not refrigerate blood, as this may result in inaccurate subset percentages (8).

9. PROCEDURE

1. Determine the absolute white blood cell count and the absolute lymphocyte count. If the white cell count is $> 10 \times 10^9$ cells / L, dilute blood in phosphate-buffered saline to obtain approximately 5×10^9 cells / L (5×10^8 cells / μ L). In cases where an automated instrument does not provide an absolute lymphocyte count, this may be obtained by multiplying the number of leucocytes by the percent lymphocytes (obtained by manual or automated differential).
2. Label 12 x 75 mm polystyrene tubes with appropriate OptiClone Monoclonal Antibodies or Isotypic Control for each specimen.
3. Pipet 100 μ L of well mixed, anticoagulated whole blood (or prediluted sample) to the bottom of each tube. Important: Do not allow blood to remain on the inner tube walls. Briefly vortex or mechanically agitate to mix, making sure blood does not remain on the upper tube walls.
4. Add 20 μ L OptiClone CD3 FITC / CD19 PE / CD45 PE-Cy5 or 20 μ L OptiClone IgG1 FITC / IgG1 PE / CD45 PE-Cy5 to the appropriate tubes.
5. Incubate all tubes for 15 minutes at room temperature (18-25°C) in the dark.
6. Lyse red blood cells according to OptiLyse Lysing Solution package insert.
7. Analyze preparation on a flow cytometer equipped with the optical pathways required to collect FITC, PE and PE-Cy5 fluorescent emissions.



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