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OptiClone®

Monoclonal Antibodies CD3 FITC / CD4 PE

50 Tests Cat. No. 1382

For research use only

Not for use in diagnostic procedures.

1. INTENDED USE

The monoclonal antibodies, UCHT-1 and 13B8.2 bind to surface antigens present on human lymphocytes. UCHT-1 has been classified as CD3, and 13B8.2 as CD4, based on standard nomenclature (1,2).
This antibody combination is intended for simultaneous flow cytometric analysis of $CD3^+$ T cells and the $CD4^+$ helper/inducer subset of T cells.

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2. SUMMARY AND EXPLANATION

T cells are one of the major lymphocyte populations in human blood. They are characterized by expression of the cell surface antigen CD3, and are commonly divided into the helper / inducer subset, expressing the CD4 antigen, and the suppressor / cytotoxic subset, which express CD8 (3-5).
CD3 antigen is comprised of at least 5 invariant polypeptide chains (γ, δ, ε, ζ and η), and is associated with α / β or γ / δ heterodimers as part of the CD3-T cell receptor (TCR) complex (1, 5).
CD3 is expressed only on cells of T lineage such as mature T cells and a subset of thymocytes. Approximately 65-75% of lymphocytes in peripheral blood are $CD3^+$, although the percentage in children is lower and is age-related (6).
CD4 is a 60 kDa monomeric glycoproteic antigen and is expressed on the subset of T cells that function as helper / inducer cells (7). CD4 is present on approximately 45 % of normal peripheral blood lymphocytes, 80% of thymocytes, and on monocytes and some neutrophils at low density (7, 8). The CD4 antigen interacts with class II major histocompatibility complex (MHC) antigens during cell activation and is part of the human immunodeficiency virus (HIV) receptor on T cells (8-10).

3. PRINCIPLE OF THE TEST

This CD3 / CD4 formulation is optimized for flow cytometric use after lysis in a no-wash procedure.
CD3 (UCHT1) is conjugated to fluorescein isothiocyanate (FITC); CD4 (13B8.2), to R-phycoerythrin (PE); Upon excitation at 488 nm, each fluorescent dye emits light at different wavelengths, making possible the simultaneous study of CD3 and CD4 antigens respectively.
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 forward scatter representation (scattergram) permits discrimination of lymphocytes (low side scatter level and medium to high forward scatter level) from debris, and monocytes or polymorphonuclear cells. A gate may be drawn around the lymphocytes.
A fluorescent dual color representation of CD3 FITC versus CD4 PE can be analyzed and the percentage of $CD3^+CD4^+$ T lymphocyte subset be precisely determined.
The accuracy of these analysis is dependent on the correct identification of the cells of interest by light scattering properties detected by flow cytometer.

4. REAGENTS AND MATERIALS

Reagent provided: One vial containing 1.0 mL (50 tests) of OptiClone CD3 FITC / CD4 PE 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 (12).
CD4: Clone 13B8.2 was produced by the fusion of spleen cells from Balb/c mice immunized with human thymocytes with the mouse NS1 myeloma cell line.
Immunoglobulin (Ig) Structure: Each of the antibodies is 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). CD4 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).

WARNING
This reagent contains sodium azide, as 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.

5. REAGENTS AND MATERIALS REQUIRED BUT NOT PROVIDED

1. OptiClone Monoclonal Antibodies IgG1 FITC / IgG1 PE mouse negative control (Cat. No. 1388).
2. No-wash lysing reagent. For example: OptiLyse® Lysing Solution (Cat. No. 1400 or 1401).
3. Phosphate-buffered saline (0.01 M sodium phosphate, 0.145 M sodium chloride, pH 7.2).
4. Evacuated blood collection tubes with anticoagulant.
5. Polystyrene tubes (12 x 75 mm). Do not use glass tubes.
6. Pipettors and pipet tips capable of delivering 20 µL and 100 µL.
7. Vortex mixer.
8. Flow cytometer equipped with the optical pathways required to collect FITC, and PE fluorescent emissions.
9. Blood cell counter

6. 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.

7. REAGENT PREPARATION

OptiClone Monoclonal Antibodies are ready-to-use with no further dilution or preparation. Do not expose to direct light for prolonged periods.

8. REAGENT STORAGE

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

9. BLOOD SAMPLE COLLECTION

Peripheral blood should be obtained by aseptic venipuncture. A salt of EDTA (K_3 EDTA) is recommended, however ACD or heparin, may also be used.
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 fresh specimens. Do not refrigerate blood, as this may result in inaccurate subset percentages (11).

10. PROCEDURE

1. Determine the absolute white blood cell count and the absolute lymphocyte count. In case of white cell count >10 x 10^9 cells / L, dilute blood in phosphate-buffered saline to obtain approximatly 5 x 10^9 cells / L (5 x 10^3 cells / µL). 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).
   Label 12 x 75 mm tubes with appropiate Opticlone Monoclonal Antibodies or Isotypic Control names for each specimen.
2. Pipet 20 µL OptiClone CD4 FITC / CD8 PE or 20 µL OptiClone IgG1 FITC / IgG1 PE to the **bottom** of appropiated tubes.
3. Add 100 µL of anticoagulated whole blood into the bottom of each tube and briefly vortex or mechanically agitate to mix, making sure blood does not remain on the upper tube walls.
4. Incubate all tubes for 15 minutes at room temperature (18-25°C), in the dark.
5. Lyse red blood cells according to OptiLyse Lysing Solution package insert.
6. Analyze cell preparation on a flow cytometer equipped with the optical pathways required to collect FITC, and PE fluorescent emissions.

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11. FLOW CYTOMETRIC ANALYSIS

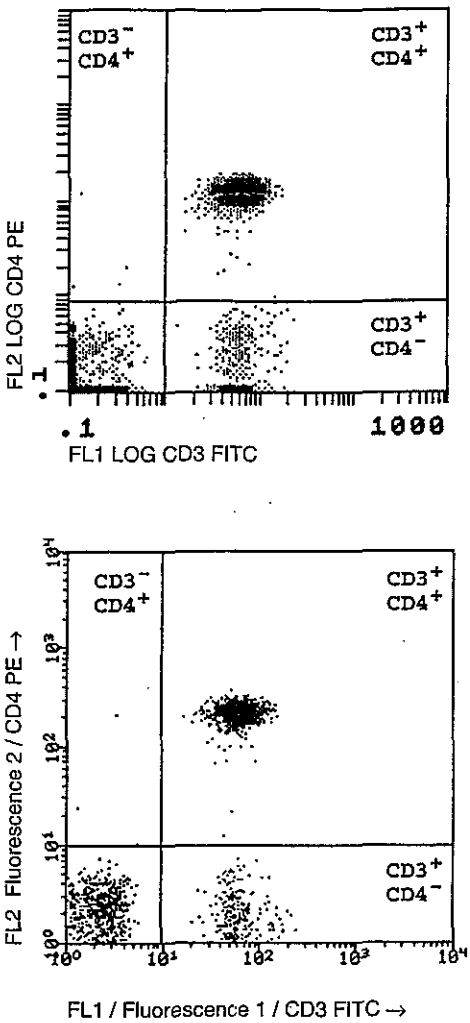
Analyze stained cells according to the instrument manufacturer's guidelines. Lymphocytes should be identified on the basis of dual-parameter analysis on side scatter (SSC) versus forward scatter (FSC) scattergram.
The determination of positivity / negativity for immunostained cells should be based on the non-specific fluorescence of the Opticlone Negative Control-stained cells.

12. LIMITATIONS

1. OptiClone Monoclonal Antibodies should be used in conjunction with an ongoing daily laboratory quality control program to monitor instrument performance and determine reference ranges for immunostained cells under the methodologic conditions employed. This should be monitored daily by a comprehensive program of standards and controls as suggested in the guideline cited above (11). Participation in interlaboratory proficiency testing programs is encouraged.
2. Reliable and reproducible results will be obtained when the procedure is carried out in accordance with package insert instructions, and with adherence to good laboratory practice, and prior to the expiration date on the vial.
3. Data interpretation should be based on the reference range established by each laboratory.
4. Test results should be considered only in the context of other pertinent clinical and laboratory information.
5. When stored at 2-8°C antibody reagents are stable until the expiration date shown on each bottle. Do not use beyond this date.
6. PE-Cy5 conjugates are recommended for use only on flow cytometers equipped with a 675 nm band pass filter.
7. Due to an unacceptable variance among the different laboratory methods for determining the absolute lymphocytes counts, an assessment of the accuracy of the method used is necessary.

13. EXPECTED RESULTS

The dual-color histograms below illustrate the expected spatial separation of peripheral blood lymphoid cellular subsets when immunostained with OptiClone Monoclonal Antibodies CD3 FITC/CD4 PE using the OptiLyse Lysing Solution and analyzed by flow cytometry.



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