OptiClone®

Monoclonal Antibodies
CD4-FITC / CD8-PE / CD3-PC5

50 Tests PN IM1650

For Research Use Only.

Not for use in diagnostic procedures.

1. INTENDED USE

This three-color monoclonal antibody combination is intended for simultaneous flow cytometric identification of the $CD3^+$ T cells and the $CD4^+$ helper / inducer, and $CD8^+$ suppressor / cytotoxic T cell subpopulations (1).

2. SPECIFICTY

CD3 antigen is comprised of at least 5 invariant polypeptide chains (γ, δ, ε, ζ, and η), and is associated with either α/β or γ/δ heterodimers as part of the CD3-T cell receptor (TCR) complex (2, 3). 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 (4).

CD4 is a 60 kDa monomeric glycoprotein and is expressed on the subset of T cells that function as helper / inducer cells (5). CD4 is present on approximately 45 % of normal peripheral blood lymphocytes, 80% of thymocytes, on monocytes, and some neutrophils at low density (5, 6). 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 (6 – 8).

The 32 kDa CD8 antigen is present on the T cell subset containing suppressor / cytotoxic cells, and is found on approximately 30% of peripheral blood lymphocytes (5, 9). CD8 is also present on the majority of natural killer (NK) cells and most thymocytes (9). It interacts with class I major MHC antigens to facilitate cellular activation (1, 6).

The UCHT1 and B9.11 mAbs were assigned to CD3 and CD8 respectively during the 1st HLDA Workshop on Human Leucocyte Differentiation Antigens, held in Paris, France, in 1982 (10, 11). The 13B8.2 mAb was assigned to CD4 during the 3rd HLDA Workshop on Human Leucocyte Differentiation Antigens, Oxford, England in 1986 (12).

3. PRINCIPLE OF THE TEST

This CD4 / CD8 / CD3 conjugated monoclonal antibodies formulation is optimized for flow cytometric use after lysis in a no-wash procedure. Upon excitation at 488 nm, each fluorescent dye emits light at different wavelengths, making possible the simultaneous study of CD4, CD8 and CD3 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 from debris, and monocytes or polymorphonuclear cells. A gate may be drawn around the lymphocytes.

A fluorescent dual color dot-plot of CD4-FITC versus CD3-PC5 can be analyzed and the percentage of $CD3^+CD4^+$ T lymphocyte subset be precisely determined. Similarly, a fluorescent dual color dot-plot of CD8-PE versus CD3-PC5 can be studied and the percentages of $CD3^+$ and / or $CD8^+$ lymphocyte subpopulations be simultaneously determined. Finally, CD4 and CD8 double positive T cells can be monitored using the fluorescent dual color dot-plot of CD8-PE versus CD4-FITC.

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. REAGENT

OptiClone Monoclonal Antibodies
CD4-FITC / CD8-PE / CD3-PC5
PN IM1650 – 1 mL – 20 µL / test

Clone #1: 13B8.2
Specificity: CD4
Hybridoma: NS1 x Balb/c
Immunogen: Human thymocytes
Isotype: IgG1
Species: Mouse
Source: Ascites fluid
Purification: Ion exchange or affinity chromatography
Conjugation: FITC (Fluorescein isothiocyanate)
Molar Ratio: FITC / protein: 3 – 6
Fluorescence: FITC (Green)
Excites at 468 – 509 nm
Emits at 504 – 541 nm

Clone #2: B9.11
Specificity: CD8
Hybridoma: NS1 x Balb/c
Immunogen: Human HLA A2 cytotoxic T-cell clone
Isotype: IgG1
Species: Mouse
Source: Ascites fluid
Purification: Ion exchange or affinity chromatography
Conjugation: PE (R-phycoerythrin)
Molar Ratio: PE / protein: 0.5 – 1.5
Fluorescence: PE (Orange)
Excites at 486 – 580 nm
Emits at 568 – 590 nm

Clone #3: UCHT1
Specificity: CD3
Hybridoma: NS1 x Balb/c
Immunogen: Human peripheral blood lymphocytes
Isotype: IgG1
Species: Mouse
Source: Ascites fluid
Purification: Ion exchange or affinity chromatography
Conjugation: PC5 (Phycoerythrin-Cyanine 5)
Molar Ratio: PC5 / protein: 0.5 – 1.5
Fluorescence: PC5 (Deep Red)
Excites at 486 – 580 nm
Emits at 660 – 680 nm

5. MATERIALS REQUIRED BUT NOT PROVIDED

1. No-wash lysing reagent. (e.g: OptiLyse® Lysing Solution, PN A11894 or IM1400, Beckman Coulter)
2. Conjugated isotypic control calibrated for "no-wash" procedures (e.g., OptiClone® IgG1-FITC / IgG1-PE / IgG1-PC5 mouse Isotypic control (PN IM1672, Beckman Coulter).
3. Phosphate-buffered saline (0.1 M sodium phosphate, 0.145 M NaCl).
4. Whole blood collection tubes with anti-coagulant (EDTA is recommended).
5. 12 x 75 mm test tubes.
6. Pipettors and pipet tips capable of delivering 20 µL and 100 µL.
7. Timer.
8. Vortex mixer.
9. Flow cytometer.
10. Cell counter or haemocytometer.

6. STATEMENT OF WARNINGS

1. This reagent contains 0.1% sodium azide. Sodium azide under acid conditions yields hydrazoic acid, an extremely toxic compound. Azide compounds should be flushed with running water while being discarded. These precautions are recommended to avoid deposits in metal piping in which explosive conditions can develop. If skin or eye contact occurs, wash excessively with water.
2. All blood samples must be considered as potentially infectious and must be handled with care (in particular: the wearing of protective gloves, gowns and goggles).
3. Never pipet by mouth and avoid contact of specimens, samples, or reagents with skin, mucous membranes, and eyes.
4. Do not use the reagent beyond expiry date.
5. Incubation times or temperatures other than those specified may give erroneous results.
6. Avoid microbial contamination of the reagent or erroneous results may occur.
7. Do not expose reagents to strong light during storage or incubation.
8. Use good laboratory practices when handling this reagent.

7. STORAGE CONDITION AND STABILITY

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.

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8. REAGENT PREPARATION

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

9. BLOOD SAMPLE COLLECTION

Venous blood samples must be taken using sterile tubes. A salt of EDTA is recommended as anticoagulant, however ACD and heparin may also be used.
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.
The sample must be analyzed within 24 hours of venipuncture and should be homogenized by gentle agitation prior to taking the test sample.

10. SAMPLE PREPARATION

Determine the absolute white blood cell count. In case of white cell count > 10 x 10^9 cells/L, dilute blood in phosphate-buffered saline to obtain approximately 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).

11. PROCEDURE

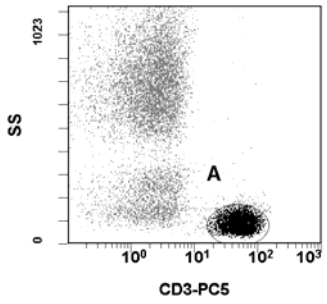
1. Label 12 x 75 mm tubes with appropiate OptiClone Monoclonal Antibodies or Isotypic Control names for each specimen.
2. Pipet 20 µL OptiClone CD4-FITC / CD8-PE / CD3-PC5 or 20 µL OptiClone IgG1-FITC / IgG1-PE / IgG1-PC5 to the bottom of appropiated tubes.
3. Add 100 µL of anticoagulated whole blood 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, PE and PC5 fluorescent emissions.

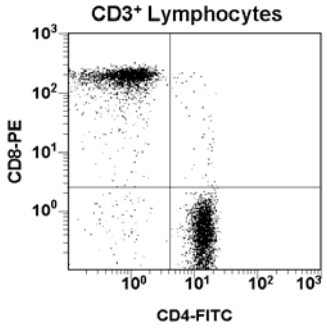
12. FLOW CYTOMETRIC ANALYSIS

Analyze stained cells according to the instrument manufacturer's guidelines. Lymphocytes should be identified on the basis of their low forward and side scatters on a forward scatter (FS) *versus* side scatter (SS) dual-parameter histogram. The determination of positivity/negativity for immunostained cells should be based on the non-specific fluorescence of the isotypic control-stained cells.

13. EXAMPLE OF DATA

The histograms below illustrate the immunostaining of peripheral blood lymphoid cellular populations with OptiClone Monoclonal Antibodies CD4-FITC / CD8-PE / CD3-PC5 after lysis with OptiLyse C Lysing Solution and analyzed by flow cytometry.
Acquisition was with a COULTER EPICS XL™ flow cytometer. Analysis is with the CXP Software.





14. LIMITATIONS

1. Reliable and reproducible results will be obtained when the procedure is carried out in accordance with package insert instructions,
2. 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.
3. Results obtained with flow cytometry may be erroneous if the instrument is misaligned, compensation spillovers are not correctly compensated or the gates are improperly set. The stability of blood samples is quite variable.
4. Do not refrigerate blood, as this may result in erroneous subset percentages.
5. PC5 conjugates are recommended for use only on flow cytometers equipped with a 675 nm band pass filter.

15. REFERENCES

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16. TRADEMARKS

For additional information in the USA,
call 800-526-7694.
Outside the USA, contact your local Beckman Coulter representative.
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