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OptiClone®

Monoclonal Antibodies CD45 FITC / CD14 PE

50 Tests Cat. No. 1387

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

1. INTENDED USE

The monoclonal antibodies Immu19.2 and RMO52 bind to surface antigens present on human leucocytes. Immu19.2 recognizes the CD45 specificity and RMO52 has been classified as CD14 based on standard nomenclature (1, 2). This antibody combination is intended for simultaneous flow cytometric analysis of $CD45^+$ leucocytes and $CD14^+$ monocytes.

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2. SUMMARY AND EXPLANATION

Lymphoid and hematopoietic cell differentiation is characterized by expression of certain cell surface molecules (antigens) at particular stages of maturation using monoclonal antibodies of well-defined specificities (3). Knowledge of these specific reaction patterns facilitates definition and quantitation of cell subpopulations.
The CD45 family of transmembrane glycoproteins is present (in cell-type-related pattern) on all human leucocytes, but not on mature erythrocytes (4). At least 4, and possibly as many as 8, isoforms of CD45 exist that differ in relative molecular mass (M_r) from 180-220 (4). Different isoforms may be produced by alternative splicing of the mRNA in cells (4). The sensitivity of gel electrophoresis and the small differences in M_r of certain CD45 isoforms limit the ability to readily resolve these proteins, therefore immunophenotypic analysis of cells using monoclonal antibodies reactive with various isoforms of CD45 have become important. Monoclonal antibodies that react with a putative "backbone" structure common to all isoforms are referred to as CD45, while those restricted to protein segments (usually in the N-terminal region) may be referred to as CD45RA, CD45RB, or CD45R0 (1, 4, 5). The clone Immu19.2 reacts with the backbone structure common to all known isoforms of CD45 and is therefore considered as pan CD45.
Functionnally, proteins of the CD45 family are enzymes of the protein tyrosine phosphatase group (6). It has been shown that lymphocytes exhibit a reciprocal balance between the phosphatase activity of CD45 and the tyrosine kinase activity of *src* gene products such as Lck (7, 8). Through this mechanism, CD45 is involved in transmembrane signal transduction (4, 7, 8).
CD14 is a glycosyl-phosphatidylinositol anchored membrane protein of M_r 53 and is expressed strongly on monocytes and macrophages and dimly on polymorphonuclear leucocytes (2, 9, 10). CD14 is involved in bacterial lipopolysaccharide (LPS) induced cellular activation and, in fact, may be a receptor for LPS (9)

3. APPLICATIONS

Identification and enumeration of $CD45^+$ leucocytes in peripheral blood have proven useful in flow cytometric discrimination of lymphocyte and non-lymphocyte leucocytic cell populations from erythrocytes (11). CD45 therefore aids in determination of the level of non-leucocytic "contamination" of the lymphoid region as determined by light scatter in conjunction with fluorescence.
In addition to its qualitative presence on all normal leucocytes, CD45 binds to polymorphonuclear leucocytes (PMNs) and monocytes in a quantitatively different amount than lymphocytes (1, 4). This quantitative difference is useful to help discriminate the populations of PMN, monocytes, and lymphocytes using fluorescence and light scatter properties.
Most peripheral blood monocytes express CD14 (2, 9). This attribute discriminates monocytes from lymphocytes, and is therefore helpful in determining the proportion of leucocytes within the "lymphoid" light scatter region that are monocytes. On this basis, performance of dual-color immunostaining with CD45 / CD14 has been suggested as a useful tool in immunophenotypic analysis of peripheral blood lymphocytes (11).
Since CD14 may be found on cells other than monocytes, albeit at decreased density (2) and CD45 is also dimly expressed on PMNs (1, 4) the use of combined CD45 / CD14 antibodies ensures enumeration of only leucocytes, and the proportion of lymphocytes and non-lymphocytes within the analytical light scatter region.

4. PRINCIPLES OF THE TEST

This CD45 / CD14 formulation is optimized for flow cytometric use after lysis in a no-wash procedure, and is one of the OptiClone Monoclonal Antibody products from Immunotech. After the whole blood sample is incubated with the fluorescent antibodies, the red blood cells are lysed. The processed sample is then analyzed by flow cytometry.
Upon excitation at 488 nm, each fluorochrome emits light at a different wavelength, making possible simultaneous analysis of CD45 and CD14 antigens on the surface of human leucocytes.

5. REAGENT SPECIFICATIONS

CD45 FITC / CD14 PE: 50 tests, 1.0 ml. Ready-to-use.
CD45: Clone Immu19.2 was produced by the fusion of spleen cells from Balb/c mice immunized with Epstein-Barr virus-transfected human B cells and the murine X63.Ag.8653 myeloma cell line. This monoclonal antibody is thought to recognize all isoforms of CD45 expressed on human leucocytes.
CD14: Clone RMO52 was produced by the fusion of spleen cells from Balb/c mice immunized with human monocytes with the mouse SP2/0 myeloma cell line.
Immunoglobulin (Ig) Structure: Both antibodies are comprised of mouse kappa light chains, with gamma 1 heavy chains for the Immu19.2 antibody and gamma 2a for the RMO52 antibody.
Purification: From ascites fluid by affinity chromatography.
Buffer: Phosphate-buffered saline: 0.01 M sodium phosphate, 0.145 M sodium chloride) plus 2.0 mg/ml bovine serum albumin, pH 7.2.
Preservative: 0.1% (w/v) sodium azide.
Conjugation: CD45 FITC, conjugated with fluorescein isothiocyanate (4-6 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. Phycoerythrin is excited at 488 nm, with its emission peak near 575 nm (orange-red).

6. STATEMENT OF WARNINGS

1. This product is for research use only. Not for use in diagnostic procedures.
2. This preparation contains sodium azide, which under acidic conditions forms hydrazoic acid and may be hazardous. Disposal into sewage systems should be accompanied with large quantities of running water to avoid dangerous buildup in metal piping and potential explosions.
3. 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 is recommended, but the use of other anticoagulants, including ACD and heparin, have been addressed (11). 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. MATERIAL PROVIDED

OptiClone Monoclonal Antibodies CD45 FITC / CD14 PE: 50 tests. 1.0 ml.

11. MATERIAL REQUIRED BUT NOT PROVIDED

1. OptiClone Monoclonal Antibodies mouse IgG1 FITC / IgG2a PE Isotypic Negative Control (Cat. No1389).
2. No-wash lysing reagent. For example: Opti-Lyse® Lysing Solution (Cat. No.1400 or 1401).
3. Phosphate-buffered saline (0.01 M sodium phosphate, 0.145 M sodium chloride), pH7.2.
4. Evacuated blood collection tubes with anticoagulant .
5. Plastic 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.
9. Blood cell counter.

12. PROCEDURES

1. Determine the absolute white blood cell count and the absolute lymphocyte count. If the white cell count is > 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 multipling the number of leucocytes by the percent lymphocytes (obtained by manual or automated differential).
2. Label 12 x 75 mm tubes with appropriate OptiClone Monoclonal Antibodies or Isotypic Control names for each specimen.
3. Pipet 100 µl of anticoagulated whole blood into the **bottom** of each tube. Do not allow blood to remain on inner walls of tubes.
4. Add 20 µl OptiClone CD45 FITC / CD14 PE or 20 µl OptiClone IgG1 FITC / IgG2a PE Isotypic Control to the appropriated tubes

and briefly vortex or mechanically agitate to mix, making sure the specimen does not remain on the upper tube walls.
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 or the package insert of any other no-wash lysing solution you may use.
7. Analyze cell preparation by flow cytometry.

13. FLOW CYTOMETRIC ANALYSIS

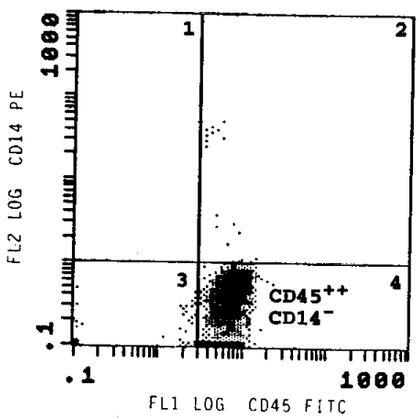
Analyze stained cells according to the instrument manufacturer's guidelines for dual-color analysis. Lymphocytes should be identified on the basis of dual - parameter forward angle and perpendicular light scatter histograms. The determination of positivity / negativity for immunostained cells should be based on the non-specific fluorescence of the OptiClone Monoclonal Antibodies IgG1 FITC / IgG2a PE Isotype Control-stained cells.

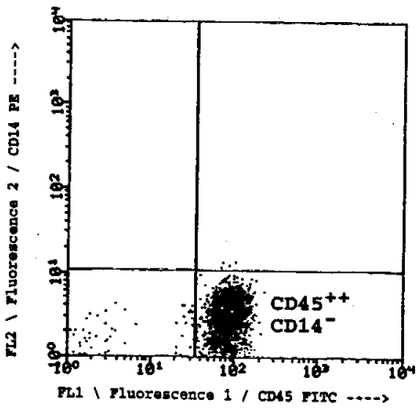
14. LIMITATIONS

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 suggested in the already quoted Guideline (11). Partipation 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, 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. When stored at 2-8°C, antibody reagents are stable until the expiration date shown on each bottle. Do not use beyond this date.
5. Due to an unacceptable variance amount in the different laboratory methods for determining the absolute lymphocyte counts, an assesment of the accuracy of the method used is necessary (12).

15. EXPECTED VALUES

Each laboratory should establish reference ranges for the local population of healthy persons, as well as for age, sex, ethnic, and other potential regional differences.
The dual-color histograms below illustrate the expected spatial separation of peripheral blood lymphoid cells when immunostained with OptiClone Monoclonal Antibodies CD45 FITC / CD14 PE, lysed with OptiLyse Lysing Solution and analyzed by flow cytometry.





16. PERFORMANCE CHARACTERISTICS

The CD45 family of transmembrane glycoproteins is present in a cell-type-related pattern on all human leucocytes, but not on mature erythrocytes (1, 4). Multiple isoforms of CD45 exist that differ in M_r from 180-220. These isoforms may be produced by alternative splicing of the mRNA in cells (1, 4). The sensitivity of gel electrophoresis and the small differences in M_r of certain CD45 isoforms limits the ability to readily resolve these proteins. Immunophenotypic analysis of cells using monoclonal antibodies reactive with various isoforms of CD45 have therefore become important. Monoclonal antibodies that react with a putative "backbone" structure common to all isoforms are referred to as CD45, while those restricted to protein segments (usually in the N-terminal region) may be referred to as CD45RA, CD45RB, or CD45R0 (1, 4, 5). The clone Immu19.2 reacts with the backbone structure common to all known isoforms of CD45 and is therefore a pan CD45.
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17. REFERENCES

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