

6602622 - 100 tests

PN 178466-A



MONOCLONAL ANTIBODY

For In Vitro Diagnostic Use

INTENDED USE

COULTER CLONE MY4 (CD14) is a murine monoclonal antibody reagent. In conjunction with a fluorescent label, it is used to identify and enumerate the percentage of MY4+ monocytes in whole blood or mononuclear cell preparations by fluorescence microscopy or flow cytometry.

SUMMARY AND EXPLANATION

MY4, a murine monoclonal antibody, is a member of CD14, a heterogeneous cluster of monoclonal antibodies recognizing mostly mature monocytes.^{1,2} The molecular weight of the antigen recognized by MY4 antibody has been reported as 55 kd.¹ Although Mo2 (CD14), a similar monoclonal antibody, appears to react with the same 55 kd glycoprotein, MY4 and Mo2 are specific for different epitopes.²

MY4 antibody has been shown to be strongly fluorescent with approximately 85% of peripheral blood monocytes and weakly fluorescent with approximately 28% of peripheral blood granulocytes.³ MY4 antibody defines a myeloid differentiation antigen in that the antigen is not detected on myeloid precursor cells and appears at a distinct stage of monocyte differentiation (promonocyte).³

PRINCIPLES OF TEST

This test depends on the ability of a monoclonal antibody to bind to the surface of cells expressing discrete antigenic determinants. COULTER CLONE monoclonal antibody MY4 is a murine monoclonal antibody specific for a cell surface antigen. Specific cell staining is accomplished by incubating peripheral blood cells with the monoclonal antibody, washing the cells to remove unbound antibody, and then incubating the cells with fluorescein-conjugated goat antiserum to mouse immunoglobulin. The percentage of positively-stained monocytes is determined by either fluorescence microscopy or flow cytometry. Isotypic controls and a second antibody control are used to assess nonspecific background fluorescence. (Label of isotypic control must correspond to label of monoclonal antibody.)

REAGENTS

COULTER CLONE Monoclonal Antibody:

MY4: Coulter PN 6602622 - 100 tests (0.5 mL)

CLONE: 322A-1 (MY4) was derived from the hybridization of mouse P3/NS1/1-AG4-1 myeloma cells with spleen cells from BALB/c mice immunized with human acute myelomonocytic leukemia cells.³

Ig CHAIN COMPOSITION: Mouse IgG2b heavy and Kappa light chains.

CYTOTOXICITY: Complement dependent.

SOURCE: Mouse ascites fluid or conditioned culture medium.

PURIFICATION: Affinity chromatography.

REAGENT CONTENTS

The final concentration of nonantibody reagents when reconstituted to 500 µL is 0.2% BSA, 0.01 M potassium phosphate, 0.15 M NaCl, and 0.1% NaN₃.

STATEMENT OF WARNINGS

1. These reagents contain 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. This procedure requires the use of fixative (formaldehyde). Inhalation or ingestion is harmful and may be fatal. If swallowed, induce vomiting. If skin or eye contact occurs, wash excessively with water.
3. Do not use antibody beyond the expiration date on label.
4. Samples and all material coming in contact with them should be handled as if capable of transmitting infection, and disposed of with proper precautions.
5. Never pipet by mouth and avoid contact of samples with skin and mucous membranes.
6. Do not expose reagents to strong light during storage or incubation.
7. Incubation or centrifuge times or temperatures other than those specified may give erroneous results.
8. Avoid microbial contamination of reagents or incorrect results may occur.
9. Harmful if swallowed.
10. After contact with skin, wash immediately with plenty of water.

RECONSTITUTION

1. Reconstitute the lyophilized COULTER CLONE MY4, GAM-FITC or isotypic control by adding 500 µL of distilled water to each. This makes a stock solution.
2. Centrifuge the stock solutions at 100,000 x g for 10 min for optimization of staining results.
3. Prepare working solutions* for the above reagents as follows:

Vial Size	Volume of Reagents or Isotypic Control Stock Solution/Test	**Volume of PBS/Test
100 tests	5 µL	Add 195 µL

*Diluted reagent must be used the same day as prepared.

**PBS - Phosphate Buffered Saline

STORAGE CONDITIONS

1. Unreconstituted lyophilized MY4 product may be stored at 2 to 8°C until expiration date printed on the label.
2. Storage conditions of reconstituted MY4 stock solution:
 Reconstituted according to the above procedure and stored at 2-8°C.....6 months
 Reconstituted and stored according to freezing procedure below at -70°C.....1 year
3. Avoid repeated freeze/thaw cycles. This will denature the antibody protein.
4. Do not store in a self-defrosting freezer.
5. Do not expose reagents to strong light during storage or incubation.
6. All reagents should be brought to 20-25°C prior to use.
7. If all reconstituted reagent is not to be used within six (6) months, follow the Freezing Procedure below.

FREEZING PROCEDURE FOR RECONSTITUTED PRODUCTS MATERIAL SUPPLIED

COULTER CLONE Monoclonal Antibody MY4

REAGENTS REQUIRED BUT NOT SUPPLIED

Phosphate Buffered Saline (PBS): 0.145 M NaCl, 0.01 M potassium phosphate, pH 7.2, Coulter PN 6603369 PBS containing 2% heat-inactivated fetal or newborn calf serum (2 mL calf serum diluted to 100 mL with PBS)

PROCEDURE

Reconstitute the lyophilized COULTER CLONE monoclonal antibody by adding 500 µL of distilled water as described previously. Dilute the reconstituted COULTER CLONE monoclonal antibody with PBS containing 2% heat-inactivated calf serum prior to the freezing as follows:

Vial Size	*Volume of Reconstituted Monoclonal Antibody/Test	**Volume of PBS with 2% Calf Serum/Test
100 tests	For each: 5 µL	100 µL

*This volume may be frozen in multiple test volumes.

**This yields one-half the working dilution of the monoclonal antibody.

Freeze the diluted monoclonal antibody. Stable for one year when reconstituted, stabilized with a solution of fetal or newborn calf serum in PBS, and stored at -70°C. Do not freeze and thaw repeatedly. Store in aliquots.

Prior to use, allow the diluted monoclonal antibody to reach 20-25°C. Dilute each test volume with PBS without 2% calf serum and mix well. See chart for volume.**

EVIDENCE OF DETERIORATION

Any change in the physical appearance of the reagents*, or any major variation in values for control samples may indicate deterioration and the reagents should not be used. If the lyophilized material appears moist, do not use.

***Normal Appearance of Reagents**

Lyophilized - white plug
 Reconstituted - clear colorless liquid

SPECIMEN COLLECTION AND PREPARATION SPECIMEN COLLECTION

CAUTION: The stability of blood samples is quite variable. For optimal results, start the assay within 6 hours of venipuncture. Unstained, anticoagulated blood should remain at 20-25°C until processing is begun. Do not refrigerate.

Collect venous blood sample aseptically by venipuncture into VACUTAINER® tubes (Becton-Dickinson) or equivalent using an appropriate anticoagulant (EDTA is recommended). For detailed information on the collection of whole blood by venipuncture and interfering conditions, refer to "Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture 3rd Edition (H3-A3)," published by the National Committee for Clinical Laboratory Standards (1991) Villanova, PA. For each test, 100 µL of whole blood is required. Collect a sufficient amount of blood (1 to 2 mL required per tube) to run the test, control, and have autologous plasma for sample dilution, if necessary. A white blood cell count should be performed.

SAMPLE PREPARATION Whole Blood

To prepare and analyze whole blood samples (1 to 2 mL required per tube), use the appropriate COULTER Whole Blood Lysing Reagent Kit, Coulter PN 6602764-100 tests or PN 6603152-300 tests and follow the directions in the package insert for lysing and staining whole blood samples.

Mononuclear Cell Preparation MATERIALS REQUIRED BUT NOT SUPPLIED:

Flow cytometer (COULTER® EPICS® PROFILE™ or equivalent)
 OR
 Fluorescence microscope (Leitz Laborlux 12 with I-Cube filter set or equivalent)

Cell counter (COULTER STKS™ or equivalent) or hemocytometer

Low-speed, at least 450 x g, refrigerated centrifuge with swinging bucket rotor to hold 15 mL centrifuge tubes

Stopcock grease

Microscope slides (3" x 1" x 1 mm)

Coverslips (24 x 50 mm)

Distilled or deionized water

Ficoll-Paque® (Pharmacia PN 17-0840-03) or equivalent

Phosphate Buffered Saline (PBS), 0.145 M NaCl, 0.01 M potassium phosphate, pH 7.2, Coulter PN 6603369

10% Formaldehyde in PBS

PBS containing 2% heat-inactivated fetal or newborn calf serum and 0.01% NaN₃ (2 mL calf serum and 0.1 mL of a 10% NaN₃ solution diluted to 100 mL with PBS), (wash medium)

PBS containing 0.01% NaN₃ (0.1 mL of a 10% NaN₃ solution diluted to 100 mL with PBS), (resuspension medium)

Propidium iodide (Calbiochem PN 537059) dissolved at 0.01 mg/mL or 0.05 mg/mL

Acridine orange 0.005 mg/mL (Baker PN A366-3)

VACUTAINER tubes (Becton-Dickinson) or equivalent with anticoagulant (EDTA is recommended)

Siliconizing agent for glassware (Prosil®-28, PCR, Inc.)

15 mL siliconized glass conical centrifuge tubes

12 x 75 mm siliconized glass test tubes

Transfer pipets

Ice bath

Mononuclear Cell Preparation Procedure

1. Dilute 4 mL of the whole blood sample 1:2 with 2-8°C PBS in an ice bath.
2. Layer 8 mL of diluted blood over 4 mL of Ficoll-Paque in a 15 mL siliconized centrifuge tube.

NOTE: If a larger volume of cells is desired, any number of tubes of blood may be pooled into a large siliconized glass tube or flask and treated as above.

3. Centrifuge at 2-8°C at 400-450 x g for 30 min. Mononuclear cells should form a visible clean interface between the plasma and the Ficoll-Paque. (See LIMITATIONS #5.)
4. Aspirate and retain the plasma and remove the mononuclear cell layer. Place cells in a clean 15 mL siliconized centrifuge tube. Fill the tube with 12-13 mL 2-8°C PBS and gently mix the cells. Centrifuge at 2-8°C at 400-450 x g for 8 min. Aspirate carefully and discard the supernatant.
5. Resuspend cells by filling the tube with 12-13 mL 2-8°C PBS, mix gently and centrifuge at 2-8°C at 400-450 x g for 4 min. Aspirate carefully and discard the supernatant.
6. Resuspend cells by filling the tube with 12-13 mL 2-8°C PBS, mix gently and centrifuge at 2-8°C at 400-450 x g for 3 min. Aspirate carefully and discard the supernatant.
7. Resuspend cells in 2 mL of 2-8°C wash medium or PBS, mix gently and determine cell concentration using a cell counter or hemocytometer. If necessary, add 2-8°C wash medium or PBS, or centrifuge and resuspend in 2-8°C wash medium or PBS, until the final concentration of the mononuclear cell suspension is 1 x 10⁶ cells/mL.
8. Cell viability analysis is performed by one of the following two suggested methods. To ensure maximum viability, analyze stained cells promptly.

(a) Viability by Fluorescence Microscopy:

CAUTION: Results may vary due to the type of microscope, light source, age of bulb, filter assembly, and filter thickness.

9. Place a suspension of approximately 25,000 cells (25 µL of a 1 x 10⁶ cells/mL suspension) onto a microscope slide.

10. Add 10 µL of a 0.01 mg/mL solution of propidium iodide and mix gently by stirring with a pipet tip.
11. Allow to stand for 30 s. Add 10 µL of a 0.005 mg/mL solution of acridine orange. Mix gently and allow to stand for 3 s.
12. Place a 24 x 50 mm coverslip on the slide avoiding air bubbles, seal with stopcock grease, and examine by fluorescence microscopy.
13. Count 100 cells and report viability as the percent of viable cells (which appear bright green) divided by viable cells plus nonviable cells (which appear red). If viability is not at least 85%, the cell preparation should not be used.

(b) Viability by Flow Cytometry:

Use an instrument that discriminates leukocytes and measures their fluorescence on a cell-by-cell basis.

CAUTION: If the laser on the flow cytometer is misaligned or the gates are improperly set, results may be erroneous.

9. Place 1 x 10⁶ cells into a 12 x 75 mm siliconized test tube.
10. Wash one time with 1 mL 2-8°C resuspension medium centrifuging at 2-8°C at 400-450 x g for 4 min.
11. Aspirate carefully and discard the supernatant. Add three drops of 0.05 mg/mL propidium iodide. Allow to stand for 1 min.
12. Wash two times in 1 mL 2-8°C resuspension medium centrifuging at 2-8°C at 400-450 x g for 4 min.
13. Aspirate carefully and discard the supernatant. Resuspend to 1 mL. Analyze on a flow cytometer according to the instrument manual. If viability is not at least 85%, the cell preparation should not be used.

PROCEDURE FOR IMMUNOFLUORESCENCE CELL SURFACE STAINING WITH COULTER CLONE MONOCLONAL ANTIBODY

REAGENTS REQUIRED BUT NOT SUPPLIED

Phosphate Buffered Saline (PBS): 0.145 M NaCl, 0.01 M potassium phosphate, pH 7.2, Coulter PN 6603369
PBS containing 2% heat-inactivated fetal or newborn calf serum and 0.01% NaN₃ (2 mL calf serum and 0.1 mL of a 10% NaN₃ solution diluted to 100 mL with PBS), (wash medium)

PBS containing 0.01% NaN₃ (0.1 mL of a 10% NaN₃ solution diluted to 100 mL with PBS), (resuspension medium)
10% formaldehyde in PBS
Siliconizing agent for glassware (Prosil-28, PCR, Inc.)
COULTER CLONE GAM-FITC (conjugated second antibody) PN 6602159 - 100 tests (0.5 mL),
COULTER CLONE MslgG2b isotypic control, PN 6603001 - 100 tests (0.5 mL)

EQUIPMENT REQUIRED

Flow cytometer (COULTER EPICS PROFILE or equivalent) or Fluorescence microscope (Leitz Laborlux 12 with I-Cube filter set or equivalent)

Low-speed, at least 450 x g, refrigerated centrifuge with swinging bucket rotor to hold test tubes or microtiter plates (see below for sizes)

Ultracentrifuge (Beckman Airfuge or equivalent)

Vortex mixer

Ice bath

Test Tube Method

Centrifuge fittings - 12 x 75 mm test tube holders

12 x 75 mm siliconized glass test tubes

Transfer pipets

Coverslips (22 x 22 mm)

Microscope slides (3" x 1" x 1 mm)

Microtiter Plate Method

"V" bottom, vinyl, flexible, 96-well (8.5 x 12.7 cm) microtiter plates

Plastic plate covers

Centrifuge carriers for microtiter plates

REAGENT PREPARATION

Prepare the suspension of blood cells and stock solutions. (Refer to RECONSTITUTION and SPECIMEN COLLECTION AND PREPARATION sections.) An appropriate isotypic control (in this case, MslgG2b) should be run with each sample.

PROCEDURE

Test Tube Method

1. For each sample, label two 12 x 75 mm test tubes, one for the monoclonal antibody and the other for the appropriate isotypic control. Place 1 x 10⁶ cells from the mononuclear cell preparation in each test tube and centrifuge at 2-8°C at 400-450 x g for 4 min. Aspirate and discard the supernatant.
2. Add 200 µL of COULTER CLONE monoclonal antibody working solution or 200 µL of control working solution into the appropriately labeled test tubes. Vortex gently. Incubate the reaction mixtures at 2-8°C for 30-35 min.
3. Wash each reaction mixture with 1 mL of 2-8°C wash medium and centrifuge at 2-8°C at 400-450 x g for 4 min. Aspirate carefully and discard the supernatant. Gently disrupt cell pellets on the vortex. Repeat.
4. After the second wash, aspirate carefully and discard the supernatant. Add 200 µL of GAM-FITC working solution to each cell pellet. Gently disrupt cell pellets on the vortex. Incubate at 2-8°C for 30-35 min.
5. At the end of 30 min, wash three times with 1 mL of 2-8°C resuspension medium. Each time centrifuge at 2-8°C at 400-450 x g for 4 min. Aspirate carefully and discard the supernatant. Gently disrupt cell pellets on the vortex.
6. After third wash, resuspend cells by adding 1 mL of 2-8°C resuspension medium to each test tube. Transfer into appropriate containers for fluorescence microscopy or flow cytometry analysis. To ensure maximum viability, analyze stained cells promptly.

Microtiter Plate Method

1. Adjust the concentration of the mononuclear cell preparation to 1 x 10⁶ cells/200 µL with PBS and dispense that volume into duplicate microtiter plate wells, one for the monoclonal antibody and one for the appropriate isotypic control.
2. Centrifuge the microtiter plates at 2-8°C at 400-450 x g for 5 min.
3. Remove the supernatant from each well by aspiration with a Pasteur pipet having a fire-polished and slightly bent tip. Insert the pipet tip into the well only as far as the lower ledge which permits efficient removal of all supernatant without disturbing the cell pellet.
4. Disrupt each cell pellet by carefully placing the lid on the tray and gently but vigorously pressing the microtiter plate "V" bottom onto the top of a vortex at an approximate setting of 8 or 9. All areas of the plate should be moved so that they come in contact with the vortex head. Vortex mixing should continue until all cells are resuspended.
5. Add 200 µL of COULTER CLONE monoclonal antibody or control working solution into alternate wells and gently agitate. Incubate at 2-8°C for 30-35 min.
6. Centrifuge the microtiter plates at 2-8°C at 400-450 x g for 5 min.
7. Aspirate carefully and discard the supernatant. Disrupt cell pellets on the vortex mixer as in Step 4.
8. Add 200 µL of 2-8°C wash medium to each well and centrifuge at 2-8°C at 400-450 x g for 5 min.
9. Aspirate carefully and discard the wash medium. Disrupt cell pellets on the vortex.
10. Add 200 µL of GAM-FITC working solution to each well. Mix gently. Incubate at 2-8°C for 30-35 min.

11. Centrifuge the microtiter plates at 2-8°C at 400-450 x g for 5 min. Wash cell pellets twice as in Steps 8 and 9 using 200 µL resuspension medium.

Fluorescence Microscopy Analysis

1. Transfer 200 µL of each final cell suspension to test tubes containing 20 µL of 10% formaldehyde in PBS. Place one drop of fixed cells on a microscope slide. Cover with a 22 x 22 mm coverslip avoiding air bubbles. Seal with stopcock grease and examine by fluorescence microscopy promptly.
2. Count all the cells in the field. Record the number of all mononuclear cells (lymphocytes plus monocytes) by phase contrast differential. Examine the cells under fluorescent light (488 nm filter). Record the percentage of fluorescent monocytes by switching between phase and fluorescent illumination.
3. Refer to QUALITY CONTROL PROCEDURE for description of how the isotypic control is used in the analysis of fluorescence microscopy and flow cytometry results.

Interpretation of Microscopy Results

CAUTION: Results may vary due to type of microscope, light source, age of bulb, filter assembly, and filter thickness.

Counts are made of mononuclear cells (peripherally stained cells only). One hundred cells are counted and + (green) or - (not green) are enumerated.

$$\%MY4+ = \frac{\text{Number of fluorescent monocytes}}{\text{Total mononuclear cell count (+ and - cells)}} \times 100$$

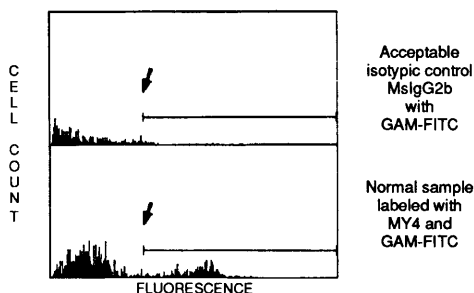
This value expresses MY4+ cells as % of the total mononuclear population.

Flow Cytometry

CAUTION: If the laser on the flow cytometer is misaligned or the gates are improperly set, results may be erroneous.

1. Analyze unfixed cells on a flow cytometer properly standardized and gated on mononuclear cells (lymphocytes plus monocytes) according to the instrument manual. To ensure maximum viability, analyze stained cells promptly.
2. The following histograms are examples of normal samples analyzed on a COULTER EPICS Profile flow cytometer and gated on mononuclear cells. The cursor (↑) was set to gate out 98±1% non-specific staining using the isotypic control. Fluorescence to the left of the cursor is non-specific staining and to the right is counted as specific staining.
3. Refer to QUALITY CONTROL PROCEDURE for description of how the isotypic control is used in the analysis of fluorescence microscopy and flow cytometry results.

Indirect Cell Surface Staining



ABSOLUTE COUNTS

To calculate Absolute Counts use the following formula:
 Absolute Counts = Total White Blood Cell Count (cells/mm³) x % Mononuclear Cells (Lymphocytes plus Monocytes) x % Positively-stained Cells /10⁴

QUALITY CONTROL PROCEDURE

A normal, apparently healthy donor should be run as a positive control to ensure proper working conditions. Normal ranges should be established within a local population of normal donors.

Nonspecific antibody Fc binding to granulocytes in a sample can be excluded by proper gating on mononuclear cells on the flow cytometer and by morphological characteristics on the microscope by phase contrast.

An appropriate COULTER CLONE isotypic control is used to negate nonspecific antibody Fc binding to lymphocytes and monocytes in each sample. By flow cytometry, the brightly fluorescent positively-stained monocyte population is measured in gates set to exclude the low level of nonspecific fluorescence. By microscopy, the nonspecific, dimly fluorescent lymphocytes and monocytes are distinguished visually from the brightly fluorescent positively-stained cells.

Nonspecific fluorescence above the background cursor (when cursor is set to gate out 98±1% nonspecific staining) is usually limited to 1-2% in normal individuals. If the background level above the cursor for any control sample is greater than 1-2%, test results may be erroneous.

LIMITATIONS

1. For optimal results, blood samples should be stained within 6 hours of collection. Retain samples in vacutainer tubes at room temperature prior to staining and analyzing. Do not refrigerate. Stored or refrigerated samples may give aberrant results. To ensure maximum viability, analyze stained cells promptly.
2. Certain patients may present special problems due to altered or very low numbers of certain cellular populations.
3. Results depend upon proper isolation of leukocytes. Prolonged contact of mononuclear cells with gradient separation medium may reduce cell viability. Remove and wash cells within five minutes after centrifugation. Prolonged exposure of whole blood samples to lytic reagents may cause white cell destruction.
4. Cells separated from whole blood by means of density gradients such as Ficoll-Paque may not have the same relative concentrations of cells as unseparated blood. This may be relatively insignificant for samples from individuals with normal white blood cell counts. In leukopenic patients, the selective loss of specific subsets may affect the accuracy of the determination.
5. Incomplete gradient separation may occur in diseases marked by changes in leukocyte size or may be due to the separation technique. Do not use the preparation if a clear-cut interfacial layer of mononuclear cells does not appear after centrifugation, or the sample has excessive erythrocytes, debris, immature myeloid cells, or granulocyte contamination. Repeat the separation procedure.
6. Cryopreserved cells must have a viability of at least 85% to be used with these reagents or incorrect results may occur.
7. Abnormal states of health are not always represented by abnormal percentages of certain leukocyte populations. An individual in an abnormal state of health may show the same leukocyte percentages as a healthy individual. Use test results in conjunction with clinical and other diagnostic data.
8. When using whole blood procedures, all red blood cells may not lyse under the following conditions: nucleated red blood cells, abnormal protein concentration or hemoglobinopathies. This may cause falsely decreased results due to unlysed red blood cells being counted as leukocytes.
9. Results obtained with fluorescence microscopy may vary due to type of microscope, light source, age of bulb, filter assembly, and filter thickness.
10. Results obtained with flow cytometry may be erroneous if the laser on the flow cytometer is misaligned or the gates are improperly set.

EXPECTED VALUES

Blood samples were collected from a population of apparently healthy males and females. This population included adults from a variety of races ranging in age from 19 to 65 years. Samples were stained with COULTER CLONE® MY4 monoclonal antibody and COULTER CLONE GAM-FITC. Normal MY4+ cell values determined by fluorescence microscopy (Leitz Laborlux 12) and flow cytometer (COULTER EPICS Profile) for mononuclear cell preparations and whole blood are given in the following table. White blood cell counts were obtained with a COULTER® S-PLUS™ IV Analyzer. Values for mononuclear cell preparations are expressed as % of the total mononuclear count and as absolute counts (cells/mm³). Values for whole blood are expressed as % of the total population (lymphocytes plus monocytes plus granulocytes) and as absolute counts (cells/mm³). Monocyte values (%) obtained for mononuclear cell preparations by phase contrast differential count (Leitz Laborlux 11 microscope) are given for comparison. These are intended as representative values only. Each laboratory should establish its own expected values for normal donors. Refer to Fluorescence Microscopy Analysis for the phase contrast differential procedure.

	MONONUCLEAR CELL PREPARATIONS			
	n	Min	Max	Mean±1 SD
%MY4+ Mononuclear Cells				
Microscopy	191	11	40	25±7
Flow Cytometry	191	10	41	22±6

MY4+ Cell Absolute Counts

Microscopy	191	307	915	606±156
Flow Cytometry	191	204	898	535±139

%Monocytes

Phase Contrast Differential Count	191	10	40	24±6
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	WHOLE BLOOD			
	n	Min	Max	Mean±1 SD
%MY4+ Cells				
Microscopy	44	4	15	8±3
Flow Cytometry	52	3	14	9±2

MY4+ Cell Absolute Counts

Microscopy	44	270	910	545±175
Flow Cytometry	52	312	882	556±145

PERFORMANCE CHARACTERISTICS SPECIFICITY

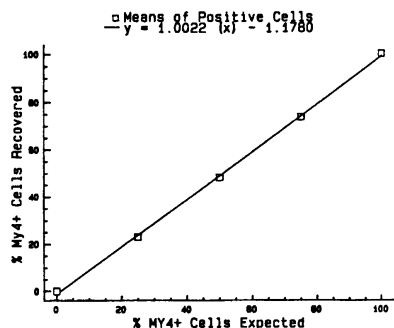
MY4 antibody has been shown to react with peripheral blood monocytes (approximately 85%) and some peripheral blood granulocytes (approximately 28%).³ MY4 antigen is expressed on 5-10% of normal bone marrow cells (primarily monocyte series cells) and on mature and some immature monocytes.^{1,3} It is present on very few (approximately 5%) uninduced promyelocytic cells from cell line HL-60, but is expressed on about 50% of two types of differentiated HL-60 cells.³ MY4 antigen is not expressed by lymphocytes, erythrocytes, platelets, or myeloid colony forming cells.³

Refer to QUALITY CONTROL PROCEDURE for description of how to control for nonspecific staining by COULTER CLONE MY4 monoclonal antibody.

LINEARITY

Dilutions were made using a mixture of a monocyte cell line (PB32) and a T cell line (PB44) which shows no response to the MY4 antibody to represent 100, 75, 50, 25, and 0 % monocytes in a standard reaction volume containing 10⁶ total cells/mL. Cells were stained with

COULTER CLONE MY4 monoclonal antibody and COULTER CLONE GAM-FITC and analyzed by flow cytometry.



Outside the US, contact your local Beckman Coulter Representative.

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PRECISION

Within day

Thirty-one replicate measurements were performed for each of three levels of monocyte cell concentrations by a COULTER EPICS PROFILE flow cytometer on the same day. A monocyte cell line (PB32) was diluted with a T cell line (PB44) which shows no response to the MY4 antibody to obtain the different concentrations. Cells were stained with COULTER CLONE MY4 monoclonal antibody and COULTER CLONE GAM-FITC. Values are expressed in terms of % of the total cell count.

Level	Mean % MY4+	±1 SD	% CV
1	5	1	20
2	11	1	9
3	24	4	17

Interlab

Studies were performed on the same day by three separate laboratories using different flow cytometers and fluorescence microscopes. Thirty-one replicate measurements were made on each instrument. Blood samples from one normal human donor were used for all measurements. Samples were stained with COULTER CLONE MY4 monoclonal antibody and COULTER CLONE GAM-FITC. Values are expressed in terms of % of the total mononuclear count.

FLOW CYTOMETRY

Lab	Instrument	Mean% MY4	±1 SD	%CV
1	EPICS Profile	16	2	12
2	EPICS® C	13	1	8
3	EPICS Profile	17	2	12

FLUORESCENCE MICROSCOPY

Lab	Mean% MY4+	±1 SD	%CV
1	15	4	27
2	13	2	15
3	16	3	19

SELECTED REFERENCES

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- McMichael AJ, ed.: Leukocyte Typing III, White Cell Differentiation Antigens: 1987. Oxford: Oxford University Press, p. 586-589, Appendix E.
- Griffin JD, Ritz J, Nadler LM, and Schlossman SF:1981. Expression of myeloid differentiation antigens on normal and malignant myeloid cells. J. Clin. Invest. 68:932-941.

PRODUCT AVAILABILITY

COULTER CLONE MY4 Monoclonal Antibody
PN 6602622 - 100 tests (0.5 mL)

For additional information in the US, call 1-800-526-7694.