## MONOCLONAL ANTIBODY

**For In Vitro Diagnostic Use**

### INTENDED USE

CYTO-STAT/Coulter Clone Mo2-RD1 or Mo2-FITC (CD14) is a fluorescent murine monoclonal antibody reagent used to identify and enumerate the percentage of Mo2+ monocytes in whole blood by flow cytometry.

### SUMMARY AND EXPLANATION

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

Mo2 antibody has been shown to react with approximately 58-84% of peripheral blood monocytes. Mo2 antibody defines a myeloid differentiation antigen in that the antigen is not detected on myeloid precursor cells including promyelocytes and appears at a distinct stage late in myeloid-monocyte-macrophage differentiation (monocyte).

### PRINCIPLES OF TEST

This test depends on the ability of a monoclonal antibody to bind to the surface of cells expressing discrete antigenic determinants. CYTO-STAT/Coulter Clone Mo2-RD1 or Mo2-FITC is a murine monoclonal antibody specific for a cell surface antigen. Specific cell staining is accomplished by incubating whole blood with the CYTO-STAT/Coulter Clone Mo2-FITC or Mo2-RD1. Red blood cells are lysed and the remaining white blood cells are analyzed by flow cytometry using monocytes gates. The percentage of positively-stained monocytes is determined for each sample. A duplicate whole blood sample stained with CYTO-STAT/Coulter Clone MsIgM-RD1 or MsIgM-FITC isotypic control is used to assess nonspecific background fluorescence. (Label of isotypic control must correspond to label of monoclonal antibody.)

### REAGENTS

See table above.

### REAGENT CONTENTS

- **Mo2-FITC:** The antibody concentration is 0.55 µg/test.
- **Mo2-RD1:** The antibody concentration is 1.1 µg/test.

The final concentration of nonantibody reagents in 0.5 mL (1 vial) is 0.2% BSA, 0.01 M potassium phosphate, 0.15 M NaCl, 0.1% NaN3, and stabilizers.

### STATEMENT OF WARNINGS

1. This reagent contains sodium azide. Sodium azide under acidic 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. Do not use antibody beyond the expiration date on label.

3. Samples and all material coming in contact with them should be handled as if capable of transmitting infection, and disposed of with proper precautions.

4. Do not pipe by mouth and avoid contact of samples with skin and mucous membranes.

5. Do not expose reagents to strong light during storage or incubation.

6. Incubation or centrifuge times or temperatures other than those specified may give erroneous results.

7. Avoid microbial contamination of reagents or incorrect results may occur.

8. Harmful if swallowed.

9. After contact with skin, wash immediately with plenty of water.

### REAGENT PREPARATION

None. CYTO-STAT/Coulter Clone monoclonal antibodies are used directly from the vial with no dilution or centrifugation necessary.

### STORAGE CONDITIONS

This reagent is stable for the dating period shown on the label when stored at 2-8°C. Avoid freezing and exposure to light. All reagents should be brought to 20-25°C prior to use.

### EVIDENCE OF DETERIORATION

Any change in the physical appearance of the reagents*, or any major variation in values obtained for control samples may indicate deterioration and the reagents should not be used.

*Normal Appearance of Reagents

- **RD1 labeled:** clear colorless to pinkish liquid
- **FITC labeled:** clear colorless to yellowish liquid

### SPECIMEN COLLECTION AND PREPARATION

**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 a blood collection tube 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 (H3), Approved Edition" published by the Clinical and Laboratory Standards Institute. 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.

### PROCEDURE FOR IMMUNOFLOURESCENCE CELL SURFACE STAINING WITH CYTO-STAT/Coulter Clone Monoclonal Antibody

### MATERIALS SUPPLIED

- **CYTO-STAT/Coulter Clone Mo2-RD1 Monoclonal Antibody**
  - PN 6604500 - 50 tests (0.5 mL)
  - OR
  - PN 6604114 - 50 tests (0.5 mL)

### MATERIALS REQUIRED BUT NOT SUPPLIED

- Erythrocyte Lytic Reagent: CYTO-STAT/Coulter Clone Mo2-RD1 Monoclonal Antibody
  - PN 6604114 - 50 tests (0.5 mL)
  - OR
  - PN 6604500 - 50 tests (0.5 mL)

- COULTER IMMUNOPREP Reagent System for COULTER Q-PREP Workstation, PN 7546946 - 100 tests
  - Diluent (if necessary) Autologous plasma
  - OR
  - COULTER IMMUNOPREP Reagent System for COULTER MULTI-Q-PREP or TQ-Prep Workstation, PN 7546999 - 300 tests
  - Diluent (if necessary) Autologous plasma

- Whole Blood Lysing Reagent Kit, PN 8602764 - 100 tests, PN 8603152 - 300 tests
  - Diluent (if necessary) Phosphate Buffered Saline (pH 7.2), PN 8603369

- CYTO-STAT/Coulter Clone MsIgM-RD1 Isotypic Control, PN 6604117 - 50 tests (0.5 mL)
  - OR
  - PN 6604500 - 50 tests (0.5 mL)

- CYTO-STAT/Coulter Clone MsIgM-FITC Isotypic Control, PN 6603369
  - OR
  - PN 6604117 - 50 tests (0.5 mL)

- Siliconizing agent for glassware
  - 12 x 75 mm test tubes
Blood collection tubes with anticoagulant (EDTA is recommended)
Transfer pipets
Vortex mixer
Flow cytometer (See Instrument Requirements section)
Cell counter or hemocytometer
Micropipetors

INSTRUMENT REQUIREMENTS
Flow cytometer that provides excitation and measures emission of scatter and fluorescence as specified in the table on page 1 as applicable for your specific product. Users should refer to the manufacturer’s instrument manuals for specific instructions for setting PMT voltages and fluorescence compensation prior to analysis.

PROCEDURE
1. Optimal staining is achieved with white blood cell counts in the range of 3-10 x 10^6 cells/µL. While white blood cell counts exceeding 10 x 10^6 cells/µL require dilution, and while white blood cell counts less than 3 x 10^6 cells/µL require centrifugation and resuspension, to achieve counts in the range of 3-10 x 10^6 cells/µL. Autologous plasma is the recommended diluent when using the COULTER IMMUNOPREP Reagent System. Phosphate Buffered Saline (pH 7.2, PN 6603369) is the diluent of choice with the Whole Blood Lysing Reagent Kit.
2. An appropriate isotypic control (in this case, CYTO-STAT/COUNTER CLONE MSiG-M-RD1 or MSiG-M-FITC) should be run with each sample. For each sample, label two 12 x 75 mm test tubes, one for the monoclonal antibody and the other for the isotypic control. Add 100 µL of the venous blood sample to each test tube. Care must be taken to avoid contamination of the tops and sides of the test tubes with blood or incomplete lysis may occur.
3. Add 10 µL of the CYTO-STAT/COUNTER CLONE Mo2-RD1 or Mo2-FITC reagent or CYTO-STAT/COUNTER CLONE MSiG-M-RD1 or MSiG-M-FITC isotypic control to the labeled test tubes.
4. Vortex gently. Incubate the reaction mixtures at 20-25°C for 10-12 minutes if using the COULTER IMMUNOPREP Reagent System. Incubate the reaction mixtures at 20-25°C for 45-47 minutes if using the Whole Blood Lysing Reagent Kit.

IMPORTANT: If blood droplets remain around the top of the test tube they must be removed or nonlysed red blood cells may contaminate the final sample and skew the results. A cotton tip applicator may be used for removal.

5. Lyse the red blood cells in each test tube using the procedure recommended for the lysing method selected (COULTER IMMUNOPREP Reagent System with the COULTER Q-PREP, MULTI-Q-PREP, T-Q Prep Workstation or Whole Blood Lysing Reagent Kit).

FLOW CYTOMETRY ANALYSIS
CAUTION: If the laser on the flow cytometer is misaligned or the gates are improperly set, results may be erroneous.
1. Analyze cells on a flow cytometer properly standardized and gated on 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 XL/XL-MCL flow cytometer and gated on monocytes. The cursor (↑) was set to gate out 98 ± 1% nonspecific staining using the isotypic control. Fluorescence to the left of the cursor is nonspecific staining and to the right is counted as specific staining.
3. Refer to QUALITY CONTROL PROCEDURE for a description of how the isotypic control is used in the analysis of flow cytometry results.

Direct Cell Surface Staining

ABSOLUTE COUNTS
To calculate Absolute Counts use the following formula:

\[
\text{Absolute Counts} = \frac{\text{Total White Blood Cell Count (cells/µL) \times % Monocytes \times % Positively-stained Cells}}{10^6}
\]

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.

Non specific antibody Fc binding to granulocytes in a sample can be excluded by proper gating on monocytes on the flow cytometer.

An appropriate CYTO-STAT/COUNTER CLONE Mo2 isotypic control is used to negate nonspecific antibody Fc binding to monocytes in each sample. The brightly fluorescent positively-stained monocyte population is measured in gates set to exclude the low level of nonspecific fluorescence.

Non specific 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 blood collection 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. These reagents should not be diluted, aliquoted, or frozen. Use only as packaged.
4. These reagents are for flow cytometry use only.
5. These reagents are designed for use with whole blood preparations. They are not recommended for use with fresh or frozen mononuclear cell preparations.
6. 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 person. Use test results in conjunction with clinical and other diagnostic data.
7. 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.
8. Prolonged exposure of cells to lytic reagents may cause white blood cell destruction.
9. Results obtained with flow cytometry may be erroneous if the laser 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 CYTO-STAT/COUNTER CLONE Mo2-RD1 or CYTO-STAT/COUNTER CLONE Mo2-FITC monoclonal antibody. Normal Mo2+ cell values determined by flow cytometry (COULTER EPICS Profile or XL-MCL flow cytometer) for whole blood are given in the following table. White blood cell counts were obtained with a COULTER S-Plus IV or STKS instrument. Values are expressed as % of the total population (lymphocytes plus monocytes plus granulocytes) and as absolute counts (10^3 cells/µL). These are intended as representative values only. Each laboratory should establish its own expected values from the local population of normal donors.

WHOLE BLOOD

<table>
<thead>
<tr>
<th>n</th>
<th>Min</th>
<th>Max</th>
<th>Mean ± 1 SD</th>
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<tbody>
<tr>
<td>CYTO-STAT/COUNTER CLONE Mo2-RD1</td>
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<tr>
<td>Mo2+ Cell Absolute Counts</td>
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<td>755</td>
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<tr>
<td>CYTO-STAT/COUNTER CLONE Mo2-FITC</td>
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<td>4</td>
<td>14</td>
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<tr>
<td>Mo2+ Cell Absolute Counts</td>
<td>17</td>
<td>276</td>
<td>770</td>
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PERFORMANCE CHARACTERISTICS

SPECIFICITY
Mo2 antibody has been shown to react with 58-84% peripheral blood monocytes.1,4 Mo2 antigen is expressed on 6-16% of normal bone marrow cells, on mature monocytes, and on certain macrophages including peritoneal macrophages and cultured macrophages derived from monocytes.1 It is not present on cells cultured from cell line HL-60, but is expressed on promyelocytic HL-60 cells induced to macrophage differentiation.1,4 Mo2 antigen is not expressed by T or B lymphocytes, null cells, or granulocytes.4

CORRELATION
Normal donors were tested using CYTO-STAT/COUNTER CLONE Mo2-RD1, CYTO-STAT/COUNTER CLONE Mo2-FITC, COULTER CLONE Mo2-RD1 and COULTER CLONE Mo2-FITC monoclonal antibodies in lysed whole blood by flow cytometry. These data are given in the table below and support the premise that these reagents are equivalent in their reactivity with peripheral blood monocytes. Values are expressed in terms of % of the total population (lymphocytes plus monocytes plus granulocytes).
WHOLE BLOOD

Mean %

Reagent n Mo2+ ±1 SD %CV
CYTO-STAT/COULTER CLONE Mo2-RD1 50 6 2 30
CYTO-STAT/COULTER CLONE Mo2-FITC 17 8 3 37
COULTER CLONE Mo2-RD1 16 9 1 11
COULTER CLONE Mo2-FITC 32 7 2 28

PRECISION

Within day

Thirty-one replicate measurements were performed for each of three levels of monocyte cell concentrations by a COULTER EPICS XL-MCL flow cytometer on the same day. One normal donor was selectively depleted of monocytes positive for the Mo2 antibody. The cells were then diluted with the normal donor to obtain the different concentrations. Cells were stained with CYTO-STAT/COULTER CLONE Mo2-RD1 or CYTO-STAT/COULTER CLONE Mo2-FITC monoclonal antibody. Values are expressed in terms of % of the total population (lymphocytes plus monocytes plus granulocytes).

<table>
<thead>
<tr>
<th>CYTO-STAT/COULTER CLONE Mo2-RD1</th>
<th>Level</th>
<th>Mo2+ Mean %</th>
<th>±1 SD</th>
<th>%CV</th>
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<th>±1 SD</th>
<th>%CV</th>
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Interlab

Studies were performed on the same day by separate laboratories within Beckman Coulter using different Beckman Coulter flow cytometers. 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 CYTO-STAT/COULTER CLONE Mo2-RD1 or CYTO-STAT/COULTER CLONE Mo2-FITC monoclonal antibody. Values are expressed in terms of % of the total population (lymphocytes plus monocytes plus granulocytes).

<table>
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<tr>
<th>CYTO-STAT/COULTER CLONE Mo2-RD1</th>
<th>Lab/Instrument</th>
<th>Mo2+ Mean %</th>
<th>±1 SD</th>
<th>%CV</th>
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<tr>
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<th>Lab/Instrument</th>
<th>Mo2+ Mean %</th>
<th>±1 SD</th>
<th>%CV</th>
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SELECTED REFERENCES
