

**CYTO-STAT®/  
COULTER CLONE®  
Mo2-FITC,  
Mo2-RD1**

REF 6604114 - 50 tests

REF 6604500 - 50 tests

PN 4236037-G



	CLONE 1	CLONE 2
<b>Specificity</b>	CD14	CD14
<b>Clone</b>	116	116
<b>Hybridoma</b>	P3/NS1/1-AG4-1 x BALB/c	P3/NS1/1-AG4-1 x BALB/c
<b>Immunogen</b>	Human adherent peripheral blood mononuclear cells	Human adherent peripheral blood mononuclear cells
<b>Ig Chain</b>	IgM	IgM
<b>Species</b>	Mouse	Mouse
<b>Source</b>	Ascites fluid or conditioned medium	Ascites fluid or conditioned medium
<b>Purification</b>	Gel filtration chromatography	Gel filtration chromatography
<b>Fluorescence</b>	Excites at 486-509 nm / Emits at 504-541 nm	Excites at 486-580 nm / Emits at 568-590 nm
<b>Conjugation</b>	FITC (Fluorescein Isothiocyanate)	RD1 (Phycoerythrin)
<b>Molar Ratio</b>	FITC/Protein: 20-30	RD1/Protein: 0.5-1.5

## 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.<sup>1,2</sup> The molecular weight of the antigen recognized by Mo2 antibody has been reported as 55 kd.<sup>1,3</sup> Although MY4 (CD14), a similar monoclonal antibody, appears to react with the same 55 kd glycoprotein, Mo2 and MY4 are specific for different epitopes.<sup>2</sup>

Mo2 antibody has been shown to react with approximately 58-84% of peripheral blood monocytes.<sup>4,5</sup> 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).<sup>5,6</sup>

### 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 reagent. Red blood cells are lysed and the remaining white blood cells are analyzed by flow cytometry using mononuclear cell (lymphocytes plus monocytes) gates. The percentage of positively-stained monocytes is determined for each sample. A duplicate whole blood sample stained with CYTO-STAT/ COULTER CLONE MslgM-RD1 or MslgM-FITC isotypic control is used to assess nonspecific background fluorescence. (Label of isotypic control must correspond to label of monoclonal antibody.)

### REAGENTS

CYTO-STAT/COULTER CLONE Mo2-RD1

Monoclonal Antibody  
PN 6604500 - 50 tests (0.5 mL)

OR

CYTO-STAT/COULTER CLONE Mo2-FITC

Monoclonal Antibody  
PN 6604114 - 50 tests (0.5 mL)

**CLONE:** 116 (Mo2) was derived from the hybridization of mouse P3/NS1/1-AG4-1 myeloma cells with spleen cells from BALB/c mice immunized with human adherent peripheral blood mononuclear cells.<sup>4,5</sup>

**Ig CHAIN:** Mouse IgM heavy and Kappa light chains.<sup>4</sup>

**CYTOTOXICITY:** Complement dependent

**SOURCE:** Mouse ascites fluid or conditioned culture medium

**PURIFICATION:** Gel filtration chromatography

**CONJUGATION:** Mo2-RD1 - Phycoerythrin.  
Mo2-FITC - Fluorescein Isothiocyanate.

**MOLAR RATIO:** RD1/Protein - 0.5-1.5.  
FITC/Protein - 20-30.

**FLUORESCENCE:**

RD1 (Red) Excites at 486-580 nm  
Emits at 568-590 nm

FITC (Green) Excites at 486-509 nm  
Emits at 504-541 nm

### REAGENT CONTENTS

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% NaN<sub>3</sub>, 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. Never pipet 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 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.

### PROCEDURE FOR IMMUNOFLUORESCENCE CELL SURFACE STAINING WITH CYTO-STAT/COULTER CLONE MONOCLONAL ANTIBODY

#### MATERIAL SUPPLIED

CYTO-STAT/COULTER CLONE Mo2-RD1  
Monoclonal Antibody,  
PN 6604500 - 50 tests (0.5 mL)  
OR  
CYTO-STAT/COULTER CLONE Mo2-FITC  
Monoclonal Antibody,  
PN 6604114 - 50 tests (0.5 mL)

### MATERIALS REQUIRED BUT NOT SUPPLIED

- Erythrocyte Lytic Reagent:
1. COULTER® IMMUNOPREP™ Reagent System for COULTER Q-PREP™ Workstation, PN 7546946 - 100 tests Diluent (if necessary) Autologous plasma OR
  2. COULTER IMMUNOPREP Reagent System for COULTER MULTI-Q-PREP™ or TQ-PREP™ Workstation, PN 7546999 - 300 tests Diluent (if necessary) Autologous plasma

- OR
- Whole Blood Lysing Reagent Kit, PN 6602764 - 100 tests, PN 6603152 - 300 tests Diluent (if necessary) Phosphate Buffered Saline (pH 7.2), PN 6603369

CYTO-STAT/COULTER CLONE MslgM-RD1  
Isotypic Control,  
PN 6604117 - 50 tests (0.5 mL),

OR

CYTO-STAT/COULTER CLONE MslgM-FITC  
Isotypic Control,  
PN 6603877 - 50 tests (0.5 mL)

Siliconizing agent for glassware (Prosil®-28, PCR, Inc.)  
12 x 75 mm siliconized glass test tubes  
Blood collection tubes with anticoagulant (EDTA is recommended)  
Transfer pipets  
Vortex mixer  
Flow cytometer (COULTER EPICS® Profile or equivalent)  
Cell counter (COULTER STKS™ or equivalent) or hemocytometer

## PROCEDURE

- Optimal staining is achieved with white blood cell counts in the range of  $3-10 \times 10^6$  cells/mL. White blood cell counts exceeding  $10 \times 10^6$  cells/mL require dilution, and white blood cell counts less than  $3 \times 10^6$  cells/mL require centrifugation and resuspension, to achieve counts in the range of  $3-10 \times 10^6$  cells/mL. 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.
- An appropriate isotypic control (in this case, MslgM-RD1 or MslgM-FITC) should be run with each sample. For each sample, label two siliconized 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.
- Add 10 µL of the CYTO-STAT/COULTER CLONE Mo2-RD1 or Mo2-FITC reagent or CYTO-STAT/COULTER CLONE MslgM-RD1 or MslgM-FITC isotypic control to the labeled test tubes.
- 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.

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

## FLOW CYTOMETRY ANALYSIS

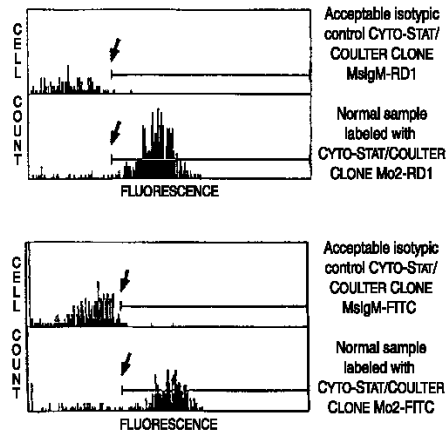
**CAUTION:** If the laser on the flow cytometer is misaligned or the gates are improperly set, results may be erroneous.

- Analyze 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.
- 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% 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.

- Refer to QUALITY CONTROL PROCEDURE for 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:  

$$\text{Absolute Counts} = \text{Total White Blood Cell Count (cells/mm}^3\text{)} \times \% \text{ Mononuclear Cells (Lymphocytes plus Monocytes)} \times \% \text{ Positively-stained Cells}/10^4$$

## 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.

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

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

- 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.
- Certain patients may present special problems due to altered or very low numbers of certain cellular populations.
- These reagents should not be diluted, aliquoted, or frozen. Use only as packaged.
- These reagents are for flow cytometry use only.
- These reagents are designed for use with whole blood preparations. They are not recommended for use with fresh or frozen mononuclear cell preparations.
- 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.

- 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.
- Prolonged exposure of cells to lytic reagents may cause white blood cell destruction.
- 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/COULTER CLONE Mo2-RD1 or CYTO-STAT/COULTER CLONE Mo2-FITC monoclonal antibody. Normal Mo2+ cell values determined by flow cytometry (COULTER EPICS Profile) for whole blood are given in the following table. White blood cell counts were obtained with a COULTER® S-PLUS IV instrument. Values are expressed as % of the total population (lymphocytes plus monocytes plus granulocytes) and as absolute counts (cells/mm<sup>3</sup>). \* These are intended as representative values only. Each laboratory should establish its own expected values from the local population of normal donors. (\*Studies in progress.)

	WHOLE BLOOD			
	n	Min	Max	Mean±1 SD
CYTO-STAT/COULTER CLONE Mo2-RD1				
%Mo2+ Cells	*	*	*	*
Mo2+ Cell Absolute Counts	*	*	*	*
CYTO-STAT/COULTER CLONE Mo2-FITC				
%Mo2+ Cells	17	4	14	8±3
Mo2+ Cell Absolute Counts	17	276	770	448±136

## PERFORMANCE CHARACTERISTICS SPECIFICITY

Mo2 antibody has been shown to react with 58-84% peripheral blood monocytes.<sup>4,5</sup> 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.<sup>1</sup> It is not present on cells cultured from cell line HL-60, but is expressed on promyelocytic HL-60 cells induced to macrophage differentiation.<sup>1,4</sup> Mo2 antigen is not expressed by T or B lymphocytes, null cells, or granulocytes.<sup>4</sup>

Refer to QUALITY CONTROL PROCEDURE for description of how to control for nonspecific staining by CYTO-STAT/COULTER CLONE Mo2-RD1 and CYTO-STAT/COULTER CLONE Mo2-FITC monoclonal antibodies.

## CORRELATION

Normal donors were tested using CYTO-STAT/COULTER CLONE Mo2-RD1, CYTO-STAT/COULTER 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). \* (\*Studies in progress.)

Reagent	WHOLE BLOOD			
	n	Mean %		
		Mo2+	±1 SD	%CV
CYTO-STAT/COULTER CLONE Mo2-RD1	*	*	*	*
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 Profile flow cytometer on the same day. A mononuclear cell preparation was prepared from one normal donor blood sample and enriched for monocytes positive for the Mo2 antibody by selective depletion of T and B lymphocytes. The cells were diluted with granulocytes negative for the Mo2 antibody 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 mononuclear count.\* (\*Studies in progress.)

Level	CYTO-STAT/COULTER CLONE Mo2-RD1		
	Mo2+ Mean %	±1 SD	% CV
1	*	*	*
2	*	*	*
3	*	*	*

Level	CYTO-STAT/COULTER CLONE Mo2-FITC		
	Mo2+ Mean %	±1 SD	% CV
1	*	*	*
2	*	*	*
3	*	*	*

### Interlab

Studies were performed on the same day by three separate laboratories within Coulter Immunology using different 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 mononuclear count.\* (\*Studies in progress.)

Lab/Instrument	CYTO-STAT/COULTER CLONE Mo2-RD1		
	Mo2+ Mean%	±1 SD	%CV
1 (EPICS Profile)	*	*	*
2 (EPICS V)	*	*	*
3 (EPICS C)	*	*	*

Lab/Instrument	CYTO-STAT/COULTER CLONE Mo2-FITC		
	Mo2+ Mean%	±1 SD	%CV
1 (EPICS Profile)	*	*	*
2 (EPICS V)	*	*	*
3 (EPICS C)	*	*	*

## SELECTED REFERENCES

- Bernard A, Boumsell L, Dausset J, Milstein C, and Schlossman SF, eds.: 1984. Leukocyte Typing: Berlin: Springer-Verlag, p. 108, 425-426, 428.
- McMichael AJ, ed.: 1987. Leukocyte Typing III, White Cell Differentiation Antigens. Oxford: Oxford University Press, p. 586-589, Appendix E.
- Todd RF, van Aghoven A, Schlossman SF, and Terhorst C.: 1982. Structural analysis of differentiation antigens Mo1 and Mo2 on human monocytes. Hybridoma 1:329-337.
- Todd RF, Nadler LM, and Schlossman SF: 1981. Antigens on human monocytes identified by monoclonal antibodies. J. Immunol. 126:1435-1442.

- Todd RF, and Schlossman SF: 1982. Analysis of antigenic determinants on human monocytes and macrophages. Blood 59:775-786.
- Todd RF, Griffin JD, Ritz BJ, Nadler LM, Abrams T, and Schlossman SF: 1981. Expression of normal monocyte-macrophage differentiation antigens on HL60 promyelocytes undergoing differentiation induced by leukocyte conditioned-medium or phorbol diester. Leukemia Res. 5:491-495.

## PRODUCT AVAILABILITY

CYTO-STAT/COULTER CLONE Mo2-RD1  
 Monoclonal Antibody  
 PN 6604500 - 50 tests (0.5 mL)  
 CYTO-STAT/COULTER CLONE Mo2-FITC  
 Monoclonal Antibody  
 PN 6604114 - 50 tests (0.5 mL)

RD1 is licensed under patent 4,520,110.

For additional information in the US, call 1-800-526-7694. Outside the US, contact your local Beckman Coulter Representative.

## TRADEMARKS

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