CYTO-STAT®/
COULTER CLONE®
Mo2-RD1/
KC56 (T-200)-FITC

REF **6603909 - 50 tests**

PN 4238087-E

	CLONE 1	CLONE 2
Specificity	CD14	CD45
Clone	116	DW124-5-2
Hybridoma	NS-1 x BALB/c	Sp2/0 x BALB/c
Immunogen	Human peripheral blood mononuclear cells	Derivative of the CEM cell line
Ig Chain	IgM	IgG1
Species	Mouse	Mouse
Source	Ascites fluid	Conditioned media
Purification	Gel filtration chromatography	Affinity chromatography
Fluorescence	Excites at 486-580 nm / Emits at 568-590 nm	Excites at 468-509 nm / Emits at 504-541 nm
Conjugation	RD1 (Phycoerythrin)	FITC (Fluorescein Isothiocyanate)
Molar Ratio	RD1/Protein: 0.5-1.5	FITC/Protein: 3-10

MONOCLONAL ANTIBODY

For In Vitro Diagnostic Use

INTENDED USE

CYTO-STAT/COULTER CLONE Mo2-RD1/KC56 (T-200)-FITC Monoclonal Antibody Reagent is a two-color fluorescent reagent comprised of two murine monoclonal antibodies conjugated to a different color fluorochrome. The reagent allows simultaneous identification and enumeration of CD14+/CD45+ [Mo2+/KC56+ (T-200)] monocytes and CD14-/CD45+ [Mo2-/KC56+ (T-200)] lymphocytes in whole blood by flow cytometry.

CYTO-STAT/COULTER CLONE Mo2-RD1/KC56 (T-200)-FITC can be used to assess the purity of the lymphocyte gate in cell surface staining procedures which use CYTO-STAT/COULTER CLONE reagents comprised of two or three-color lymphocyte and lymphocyte subset monoclonal antibodies. The reagent is not intended to select a pure lymphocyte gate, but to identify the location of the gate and to provide a measurement of nonlymphocyte contamination within the gate. Since CYTO-STAT/COULTER CLONE Mo2-RD1/KC56 (T-200)-FITC does not eliminate nonlymphocyte contamination, lymphocyte purity correction is used as a means to account for it.[1]

CYTO-STAT/COULTER CLONE Mo2-RD1/KC56 (T-200)-FITC is intended "For In Vitro Diagnostic Use" when used with CYTO-STAT/COULTER CLONE reagents labeled "For In Vitro Diagnostic Use." All other uses are "For Research Use Only. Not For Use in Diagnostic Procedures."

SUMMARY AND EXPLANATION

Mo2, a murine monoclonal antibody, is a member of CD14, a heterogeneous cluster of monoclonal antibodies recognizing mostly mature monocytes.[2,3] The molecular weight of the antigen recognized by the Mo2 antibody has been reported as 55 kd.[2,4] Although MY4 (CD14), a similar monoclonal antibody, appears to react with the same 55 kd glycoprotein, Mo2 and MY4 are specific for different epitopes.[3] The Mo2 antibody reacts strongly with >90% of peripheral blood monocytes and reacts weakly with neutrophils.[5-7] CD14 is a late myeloid differentiation antigen.[7,8] It is also a receptor for bacterial lipopolysaccharide (LPS).[6]

KC56 (T-200) recognizes members of the CD45 family of pan leukocyte antigens with molecular weights of 180, 190, 205 and 220 kd.[3,9,10] It is also known as the leukocyte common antigen (LCA). The CD45 antigen is expressed on every type of hematopoietic cell except mature erythrocytes and their immediate progenitors.[11,12] It has not been detected in differentiated nonhemapoietic tissue.[11-14]

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/KC56 (T-200)-FITC is a combination of two murine monoclonal antibodies specific for different cell surface antigens. Specific cell staining is accomplished by incubating whole blood or control cells with the dual-color CYTO-STAT/COULTER CLONE reagent. Red blood cells are lysed and the remaining white blood cells are analyzed by flow cytometry using lymphocyte and monocyte gates. The percentage of positively stained lymphocytes is determined for each of four quadrants: positive orange fluorescence only, positive green fluorescence only, dual labeled and no label.

The purity and recovery of the lymphocyte gate can be assessed using the surface staining characteristics of this monoclonal antibody. CD45 is expressed by all leukocytes, whereas the CD14 is expressed on monocytes. If there is CD14 positivity in a lymphocyte gated histogram, the lymphocyte gate is contaminated with monocytes and must be adjusted. This technique is used to identify the location of the gate. The amount of contamination is a measure of the purity of the gate at that location. Lymphocyte purity correction is used to account for the contamination of the gate in the subsequent sample tubes.

REAGENT

CYTO-STAT/COULTER CLONE
Mo2-RD1/KC56 (T-200)-FITC
PN 6603909 - 50 tests (0.5 mL)

CLONE: 116 [Mo2] was derived from the hybridization of mouse NS-1 myeloma cells with spleen cells from BALB/c mice immunized with human peripheral blood mononuclear cells.[5,7]
DW124-5-2 [KC56 (T-200)] was derived from the hybridization of mouse Sp2/0 myeloma cells with spleen cells from BALB/c mice immunized with a derivative of the CEM cell line.[6]

Ig CHAIN: Mouse IgM heavy chain and kappa light chains [Mo2][5]
Mouse IgG1 heavy chain and kappa light chains [KC56 (T-200)][6]

CYTOTOXICITY: Complement-mediated lysis [Mo2]
None by direct lysis [KC56 (T-200)]

SOURCE: Ascites fluid [Mo2]
Conditioned media [KC56 (T-200)]

PURIFICATION: Gel filtration chromatography [Mo2]
Affinity chromatography [KC56 (T-200)]

CONJUGATION: Mo2-RD1 (Phycoerythrin)
KC56 (T-200)-FITC (Fluorescein isothiocyanate)

MOLAR RATIO: FITC/protein 3-10
RD1/protein 0.5-1.5

FLUORESCENCE:
FITC (Green) Excites at 468-509 nm
Emits at 504-541 nm
RD1 (Orange) Excites at 486-580 nm
Emits at 568-590 nm

REAGENT CONTENTS

The concentration of nonantibody reagents is 0.2% BSA, 0.01 M potassium phosphate, 0.15 M NaCl, 0.1% NaN_3 and stabilizers.

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. Specimens, samples and all material coming in contact with them should be handled as if capable of transmitting infection and disposed of with proper precautions.
3. Never pipet by mouth and avoid contact of samples with skin and mucous membranes.
4. Do not use reagent beyond the expiration date on the vial label.
5. Minimize exposure of reagent to light during storage or incubation.
6. Avoid microbial contamination of reagent or erroneous results may occur.
7. Use Good Laboratory Practices (GLP) when handling this reagent.
8. Harmful if swallowed.
9. After contact with skin, wash immediately with plenty of water.

STORAGE CONDITIONS AND STABILITY

Unopened reagent is stable to the expiration date on the vial label when stored at 2-8°C. Do not freeze. Minimize exposure to light.

EVIDENCE OF DETERIORATION

Any change in the physical appearance of this reagent (clear, colorless to pink liquid) or any major variation in values obtained for control samples may indicate deterioration and the reagent should not be used.

REAGENT PREPARATION

No preparation is necessary. This CYTO-STAT/COULTER CLONE reagent is used directly from the vial. Bring reagent to 20-25°C prior to use.

SPECIMEN COLLECTION AND PREPARATION

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

Collect venous blood sample aseptically by venipuncture into a blood collection tube using an appropriate anticoagulant (EDTA is recommended).[15] 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 and have autologous plasma for sample dilution, if necessary. A white blood cell count and cell viability should be performed for each venous blood specimen using an established laboratory procedure. Recommended cell viability is ≥90% but this may be difficult to obtain with some abnormal specimens.

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

MATERIAL SUPPLIED

CYTO-STAT/COULTER CLONE
Mo2-RD1/KC56 (T-200)-FITC
PN 6603909 - 50 tests (0.5 mL)

MATERIALS REQUIRED BUT NOT SUPPLIED

Whole Blood Lysing System:

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

Optional Reagents:

COULTER® CYTO-TROL™ Control Cells

PN 6604248 - 50 tests

CYTO-COMP™ Cell Kit

PN 6607023 - (5 x 1 mL)

CYTO-COMP Reagent Kit

PN 6607021 - (4 x 0.5 mL)

12 x 75 mm test tubes

Blood collection tubes with anticoagulant (EDTA is recommended)

Transfer pipettes

Pasteur pipet

Micropipettors

Vortex mixer

Flow cytometer (COULTER EPICS® XL™/XL-MCL™, PROFILE™ or equivalent)

Cell counter (COULTER STKS™or equivalent) or hemacytometer

Cotton tip applicators

PROCEDURE

1. Optimal staining is achieved with white blood cell counts in the range of 3-10 x 10^3 cells/µL. White blood cell counts exceeding 10 x 10^3 cells/µL require dilution, and white blood cell counts below 3 x 10^3 cells/µL require centrifugation and resuspension, to achieve counts in the range of 3-10 x 10^3 cells/µL. Autologous plasma is the recommended diluent when using the COULTER IMMUNOPREP Reagent System.

Abnormal Samples

a. High White Blood Cell Count (>10 x 10^3 cells/µL)
   10-20 x 10^3: Dilute blood 1:2.
   20-30 x 10^3: Dilute blood 1:3.
   30-40 x 10^3: Dilute blood 1:5.
   40-60 x 10^3: Dilute blood 1:6.
   60-100 x 10^3: Dilute blood 1:10.
   100-200 x 10^3: Dilute blood 1:20.
b. Low White Blood Cell Count (<3 x 10^3 cells/µL) - Buffy Coat Procedure
   1) Centrifuge blood at 20-25°C at 500 x g for 5 minutes.
   2) Draw off buffy coat with a Pasteur pipet, collecting some red blood cells and some plasma to assure that all white blood cells are recovered.
   3) Completely resuspend cells by mixing several times with a Pasteur pipet.
   4) Determine cell concentration using a COULTER STKS cell counter (or equivalent) or hemacytometer.
   5) Adjust cell concentration to 10 x 10^3 cells/µL with diluent.

2. For each sample, label one 12 x 75 mm test tube and add 10 µL of the CYTO-STAT/COULTER CLONE Mo2-RD1/KC56 (T-200)-FITC.
3. 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.
4. Vortex gently. Incubate the reaction mixtures at 20-25°C for 10-12 minutes.

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 or TQ-PREP Workstation).
6. Analyze cells on a flow cytometer capable of multicolor fluorescence analysis, properly standardized and gated on lymphocytes according to the GATE PURITY AND RECOVERY ASSESSMENT PROCEDURE section. To minimize the possibility of less than optimal results, analyze stained cells promptly.
   a. Fluorescence flow cytometry readings should be collected on a log scale.
   b. Forward Scatter (FS) and Side Scatter (SS) should be collected on a linear scale.

RECOMMENDED FLOW CYTOMETRY COLLECTION PROCEDURE FOR CYTO-STAT/COULTER CLONE Mo2-RD1/ KC56 (T-200)-FITC QUALITY CONTROL PROCEDURE

Ensure the flow cytometer is properly aligned and standardized for light scatter and fluorescence intensities according to manufacturer's recommendations (refer to Instrument Manuals).

The fluorochromes fluorescein (FITC) and phycoerythrin (RD1) emit at different wavelengths, but they do have some spectral overlap which must be corrected by electronic compensation. Optimal compensation levels can be established by analyzing, on a dual-parameter histogram, donor cells with populations which stain FITC and RD1 mutually exclusive of each other. Alternatively, CYTO-COMP Cells may be stained with the appropriate fluorochrome combination from the CYTO-COMP Reagent Kit. Electronic adjustments are made to place the labeled populations into their respective fluorescence quadrants with no overlap into the dual-positive quadrant.

COULTER CYTO-TROL Control Cells stained with CYTO-STAT/COULTER CLONE Mo2-RD1/KC56 (T-200)-FITC may be used to verify compensation settings. Before samples are analyzed, an assayed control (for example, COULTER CYTO-TROL Control Cells) should be stained and analyzed to verify antibody reactivity.

GATE PURITY AND RECOVERY ASSESSMENT PROCEDURE (METHOD 1)

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

1. Collect a Forward Scatter (FS) versus Side Scatter (SS) histogram. A three-part differential may be evident. Draw a gate around the lymphocytes identified as having low FS and SS when compared to monocytes and granulocytes.
2. Collect a CD45 [KC56 (T-200)] and CD14 [Mo2] dual-parameter fluorescence histogram gated on lymphocytes. Monocyte contamination within the lymphocyte gate is represented by dual stained cells CD14+/CD45+ [Mo2+/KC56+(T-200)] in Quadrant 2 (see Figure 1). Granulocyte or red blood cell contamination within the lymphocyte gate is represented by events displayed in Quadrant 3 (see Figure 1). A fluorescence histogram displaying a lymphocyte gate without contamination is shown in Figure 2.

Figure 1. CD45 vs. CD14 histogram gated on a lymphocyte gate containing monocyte and a small amount of granulocyte contamination.

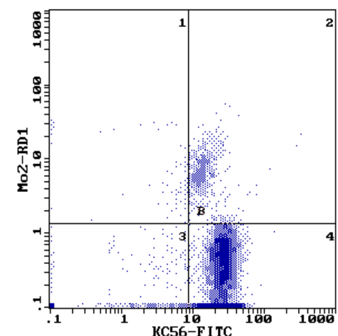
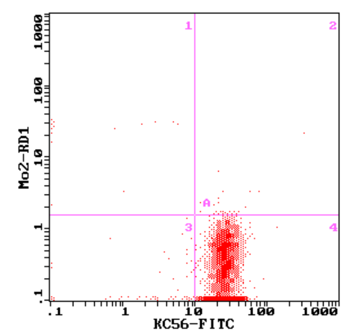


Figure 2. CD45 vs. CD14 histogram gated on a lymphocyte gate which is optimized for purity.

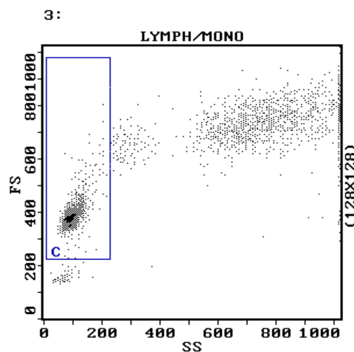


GATE PURITY AND RECOVERY ASSESSMENT PROCEDURE (METHOD 2 - THE CDC METHOD)

NOTE: This reagent is used to assess the recovery and purity of the lymphocyte gate by using the fluorescence gating technique as recommended by the Centers for Disease Control, CDC, and described in the MMWR [1997].[1] The following procedure describes the histograms used to determine the recovery and purity per the CDC guidelines.

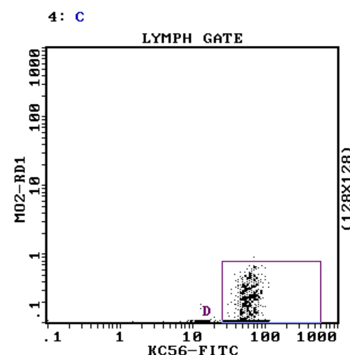
1. Collect a FS versus SS histogram and create region around the lymphocytes. Ensure that it is large enough to include all of the lymphocytes (see Region C in Figure 3).

Figure 3. FS vs. SS, ungated.



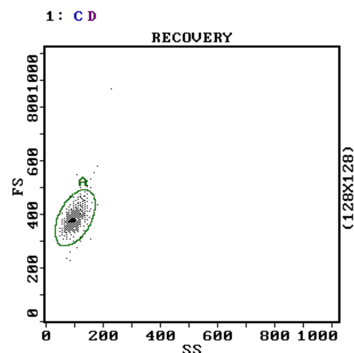
2. Collect a second histogram, CD45 [KC56 (T-200)] versus CD14 [Mo2], gated on Region C. Create a rectilinear region around the lymphocytes, the bright CD45+/CD14- population (see Region D in Figure 4).

Figure 4. CD45 vs. CD14, gated on Region C.



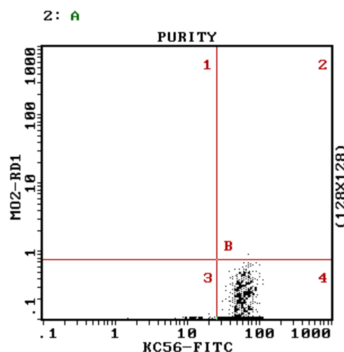
3. Collect another FS versus SS histogram, "Recovery Histogram", gated on the two lymphocyte regions, C & D. Create a smaller amorphous region around the lymphocytes (see Region A in Figure 5).

Figure 5. FS vs. SS, Recovery Histogram.



4. Collect a fourth CD45 [KC56 (T-200)] versus CD14 [Mo2] histogram, "Purity Histogram", gated on the small lymphocyte Region A created in Figure 5. Create a quadstat region on this histogram, such that quadrant 4 contains the bright CD45+/CD14- cells (see Region B4 in Figure 6).

Figure 6. CD45 vs. CD14, Purity Histogram.



5. Lymphocyte recovery is the number of events in the small lymphocyte gate displayed in Figure 5, divided by the total number of events displayed in histogram, multiplied by 100. This is simply displayed as the percentage in Region A in Figure 5.
6. The lymphocyte purity is the percentage in quadrant 4 of the "Purity Histogram." This displays the percentage of bright CD45+/CD14- cells from the small lymphocyte Region A, created in the FS vs. SS Recovery Histogram.
7. Refer to the CDC guidelines for the recommended values for purity and recovery of the gated lymphocyte population. If the recommended recovery and purity of lymphocytes cannot be achieved, redraw the gate. If minimum levels still cannot be obtained, reprocess the specimen. If this fails, request another specimen.[15]

LIMITATIONS

1. For optimal results, specimens should be stained within 6 hours of collection. Retain specimens in blood collection tubes at room temperature prior to staining and analyzing. Do not refrigerate. Refrigerated specimens may give aberrant results.
2. To minimize the possibility of less than optimal results, analyze stained cells promptly.
3. Recommended cell viability for venous blood specimens is ≥90% but this may be difficult to achieve with some abnormal specimens.
4. Prolonged exposure of cells to lytic reagents may cause white blood cell destruction and loss of cells in the population of interest.
5. 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. These unlysed red blood cells may fall within the lymphocyte gate; however, they will be negative for the CD45 (KC56) antigen.
6. This reagent is designed for use with whole blood preparations. It may be used with the lyophilized lymphocyte preparation COULTER CYTO-TROL Control Cells. It is not recommended for use with fresh or frozen mononuclear cell preparations.
7. This reagent should not be diluted, aliquoted, or frozen. Use only as packaged.
8. This reagent is for flow cytometry use only.
9. 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.
10. Certain patients may present special problems due to altered or very low numbers of certain cellular populations.
11. Results obtained with flow cytometry may be erroneous if the laser is misaligned or the gates are improperly set.
12. If using a COULTER EPICS XL/XL-MCL and the CDC Method described above for the assessment of lymphocyte purity and recovery, the "Recovery Histogram" must be the first histogram with the lymphocyte Region A as displayed in Figure 5 in order to pass the lymphocyte Region A to the following protocols in a panel.

SELECTED REFERENCES

1. Centers for Disease Control and Prevention. 1997. Revised guidelines for the performance of CD4+ T-cell determinations in persons with human immunodeficiency virus (HIV) infections. MMWR 1997;46 (No. RR-2):8-19.
2. Bernard A, Boumsell L, Dausset J, Milstein C and Schlossman SF, eds. 1984. Leukocyte Typing. New York, NY:Springer-Verlag, pp. 108, 425-426, 428.
3. McMichael AJ, Beverley PCL, Cobbold S, Crumpton MJ, Gilks W, Gotch FM, Hogg N, Horton M, Ling N, MacLennan ICM, Mason DY, Milstein C, Spiegelhalter D and Waldman H, eds. 1987. Leukocyte Typing III. Oxford, UK:Oxford University Press, pp. 586-589, 796-801, Appendix E.
4. Todd RF, van Agthoven A, Schlossman SF and Terhorst C. 1982. Structural analysis of differentiation antigens Mo1 and Mo2 on human monocytes. Hybridoma 1:329-337.
5. Todd RF, Nadler LM and Schlossman SF. 1981. Antigens on human monocytes identified by monoclonal antibodies. J Immunol 126:1435-1442.
6. Schlossman SF, Boumsell L, Gilks W, Harlan JM, Kishimoto T, Morimoto C, Ritz J, Shaw S, Silverstein R, Springer R, Tedder TF and Todd RF, eds. 1995. Leukocyte Typing V. Oxford, UK:Oxford University Press, pp. 65, 271, 778-782.
7. Todd RF and Schlossman SF. 1982. Analysis of antigenic determinants on human monocytes and macrophages. Blood 59:775-786.
8. Todd RF, Griffen JD, Ritz J, 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.
9. Newman W, Targan SR and Fast LD. 1984. Immunobiological and immunochemical aspects of the T-200 family of glycoproteins. Mol Imm 21:1113-1121.
10. Fabre JW and Williams AF. 1977. Quantitative serological analysis of a rabbit anti-rat lymphocyte serum and preliminary biochemical characterization of the major antigen recognized. Transplantation 23: 349-359.
11. Coffman RL and Weissman IL. 1981. B220: A B cell-specific member of the T200 glycoprotein family. Nature 289:681-683.
12. Dalchau R and Fabre JW. 1980. Identification with a monoclonal antibody of a predominantly B lymphocyte-specific determinant of the human leukocyte common antigen. J Exp Med 153:753-765.
13. Omary MB, Trowbridge IS and Battifora HA. 1980. Human homologue of murine T200 glycoprotein. J Exp Med 152:842-852.
14. Dalchau R, Kirkley J and Fabre JW. 1981. Monoclonal antibody to a human leukocyte-specific membrane glycoprotein probably homologous to the leukocyte-common (L-C) antigen of the rat. Eur J Immunol 10:737-744.
15. National Committee for Clinical Laboratory Standards. 1991. Procedures for the Collection of Diagnostic Blood specimens by Venipuncture. 3rd Edition (H3-A3). National Committee for Clinical Laboratory Standards, Villanova, PA.

PRODUCT AVAILABILITY

CYTO-STAT/COULTER CLONE
Mo2-RD1/KC56 (T-200)-FITC
PN 6603909 - 50 tests (0.5 mL)

RD1 is licensed under patent 4,520,110.

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

TRADEMARKS

CYTO-STAT, COULTER, COULTER CLONE, CYTO-COMP, CYTO-TROL, EPICS, IMMUNOPREP, MULTI-Q-PREP, PROFILE, Q-PREP, STKS, TQ-PREP, XL and XL-MCL are trademarks of Beckman Coulter, Inc.

Beckman Coulter, Inc.
4300 N. Harbor Blvd.
Fullerton, CA 92835
www.beckmancoulter.com

Beckman Coulter Ireland Inc.
Mervue Business Park,
Mervue, Galway,
Ireland (353 91 774068)

Pinted in USA
Made in USA

© 2004 Beckman Coulter, Inc.
All Rights Reserved.