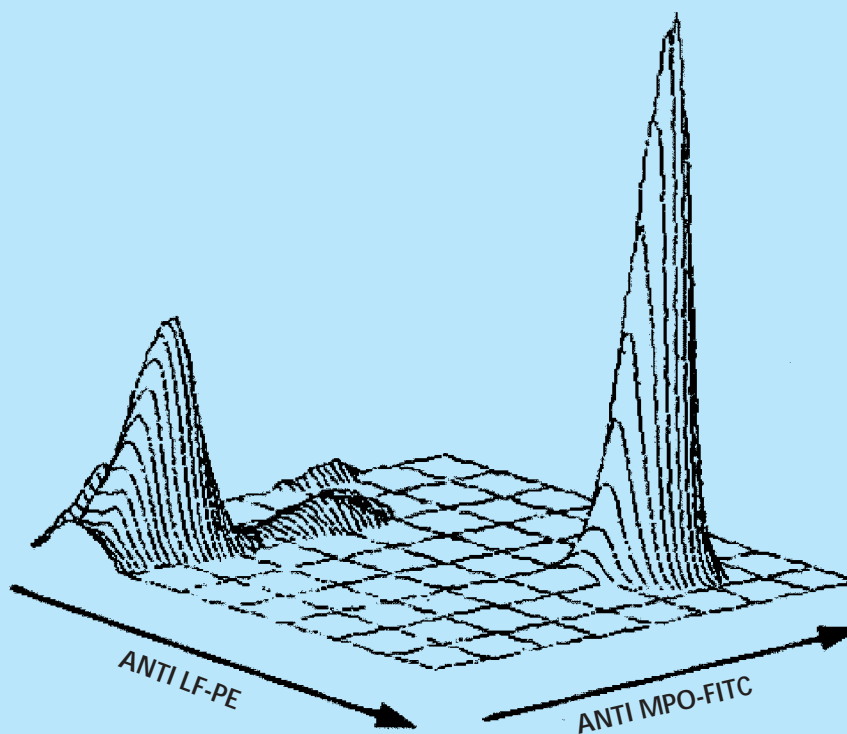


FOURTH EDITION

Additional References

Protocols for Cytokines
in Human Whole Blood

FIX & PERM[®]



FIX & PERM[®] reagents are intended for fixing cells in suspension with Reagent A and then permeabilizing the cells with Reagent B. This procedure gives antibodies access to intracellular structures and leaves the morphological scatter characteristics of the cell intact. The unique formulation of FIX & PERM[®] reagents reduces background staining and allows simultaneous addition of permeabilization medium and fluorochrome labeled antibodies.

Applications
Guide to
Intracellular
Flow
Cytometry

CALTAG
PARTNERSHIP
AN · DER · GRUB

Contents

| | |
|--|---------|
| USE OF FIX & PERM® REAGENTS | 1 |
| STAINING PROTOCOLS | 2 |
| APPLICATIONS USING FIX & PERM® | 3 |
| TYPICAL FLOW CYTOMETRIC PATTERNS | 4 |
| SCATTER PATTERNS | 4 |
| CYTOPLASMIC MYELOPEROXIDASE/CD22 – B-ALL | 4 |
| CYTOPLASMIC MYELOPEROXIDASE/CD3 – T-ALL | 5 |
| CYTOPLASMIC MYELOPEROXIDASE/LACTOFERRIN–AML, MDS, MPO DEFICIENCY ... | 5 |
| INTRACELLULAR CYTOKINE DETECTION-MURINE SPLEEN | 6 |
| CELL CYCLE ANTIGENS-KI-67 AND PCNA | 6 |
| TDT 3-COLOR USING CD45 VS. SSC GATING | 6 |
| LYSOSOMAL ANTIGENS IN PB LEUKOCYTES | 7 |
| FLUORESCENCE INTENSITY OF INTRACELLULAR VS. SURFACE ANTIGENS | 7 |
| ANTI CD65 VS. ANTI-MYELOPEROXIDASE-GRANULOCYTES | 7 |
| INTRACELLULAR IG | 8 |
| | |
| INTRACELLULAR CYTOKINES - HUMAN | |
| PROTOCOL FOR STIMULATION OF HUMAN WHOLE BLOOD & INTRACELLULAR | |
| CYTOKINE STAINING WITH FIX & PERM® | 9 |
| CYTOKINE DETECTION IN HUMAN WHOLE BLOOD - 3 COLOR ASSAYS | 10 |
| CYTOKINE DETECTION IN HUMAN WHOLE BLOOD - 4 COLOR ASSAYS | 11 |
| | |
| REAGENTS AVAILABLE FROM CALTAG | |
| FIX & PERM® CELL PERMEABILIZATION KITS | 12 |
| COMBI-IC® ANTIBODIES FOR MYELOPEROXIDASE, LACTOFERRIN, LYSOZYME, CD3, CD22 & CD68 | 12 |
| CYTO-IC® ANTIBODIES FOR MURINE CYTOKINE DETECTION | 13 |
| CYTO-IC® ANTIBODIES FOR HUMAN CYTOKINE & CHEMOKINE DETECTION | 14 |
| ANTIBODIES FOR BRDU, PCNA & HUMAN IMMUNOGLOBULINS | 15 |
| OTHER USEFUL ANTIBODIES: CD79a & p-24 | 15 |
| | |
| SELECTED REFERENCES | 16 - 17 |



The FIX & PERM® reagent system and the COMBI-IC® antibodies were developed by AN-DER-GRUB Bioresearch, Austria. These products are offered worldwide as a partnership between CALTAG and AN-DER-GRUB.

TRADEMARKS

FIX & PERM® and COMBI-IC® are shared trademarks of CALTAG and AN-DER-GRUB. CYTO-IC® is a trademark of CALTAG.

PERMEABILIZATION AND STAINING PROTOCOL

The standard procedure for intracellular staining with FIX & PERM[®] shown on the opposite page gives optimal results for most antigens. A modification using precooled absolute methanol has been shown to give better results for certain cell cycle antigens such as Ki-67 and PCNA when using FITC conjugated antibodies. The methanol step is not recommended when using R-PE conjugated antibodies.

These staining protocols are intended for use directly with the cell suspension to be analyzed.

Other lysing solutions should not be used prior to the use of FIX & PERM[®] reagents.

FLOW CYTOMETRIC ANALYSIS

FIX & PERM[®] reagents are designed for use with all commercially available flow cytometers. Alignment and compensation should be performed according to the manufacturer's instructions. Typical scatter and staining patterns for a number of applications are shown on the following pages.

BASIC AND CLINICAL RESEARCH APPLICATIONS

Flow cytometric analysis with monoclonal antibodies have been restricted primarily to cell surface molecules. Intracellular structures such as cytoplasmic or nuclear enzymes, oncoproteins, cytokines, immunoglobulins etc. were largely excluded from such studies.

Also excluded from such flow cytometric studies were cytoplasmic localizations of well established membrane molecules such as CD3 and CD22.

FIX & PERM[®] reagents allow intracellular antigen analysis with the same ease as surface antigens. The only prerequisite is the availability of suitable monoclonal antibody conjugates.

Most of the commercially available monoclonal antibody conjugates can be used with the FIX & PERM[®] reagents. Some determinants are sensitive, however, to the fixation step involved. This, and the optimal fixation time, may have to be determined experimentally for each antibody conjugate.

COMBI-IC[®] and CYTO-IC[®] reagents offered by CALTAG/AN-DER-GRUB have been determined to perform optimally when used with FIX & PERM[®].

A frequently asked question is whether indirect staining can be performed with FIX & PERM[®]. The answer is, yes, with the addition of another washing step. It is not necessary to add additional Reagent B when adding the second step reagent. Higher background staining can be encountered with indirect assays and it is usually preferable to use directly conjugated antibodies where available.

STORAGE AND STABILITY

FIX & PERM[®] reagents should be stored at room temperature. They are stable for the period shown on the package label when stored as directed. Do not use reagents if a precipitate forms or discoloration occurs. All antibody combinations should be stored in the dark at 2-8°C.

R-PE conjugates should never be frozen.

WARNING

Reagent A of the FIX & PERM[®] kit contains formaldehyde which is toxic, allergenic and a suspected carcinogen. Avoid contact with eyes, skin and clothing. All antibodies contain sodium azide as a preservative.

Staining Protocols

PROTOCOL FOR INTRACELLULAR STAINING WITH FIX & PERM

1. For each sample to be analyzed add appropriate volume of the conjugated antibody directed to the cell surface marker(s) of interest and/or the appropriate isotype control(s) to a 5 ml, 12x75 mm tube.
2. Pipette appropriate volume of adjusted cells (equivalent to 1×10^6 cells) into each tube containing the conjugated antibody or isotype control.
3. Vortex each tube gently to mix, and incubate for 15 minutes in the dark at room temperature.
4. Add 100 μ l of Reagent A (Fixation Medium) and incubate for 15 minutes at room temperature.
5. Wash once in 3 ml PBS + 0.1% NaN_3 + 5% FBS.
6. Centrifuge for 5 minutes at 300-350 x g, aspirate the supernatant, and vortex to fully resuspend the cell pellet.
7. Add 100 μ l of Reagent B (Permeabilization Medium) and the recommended volume of the FITC- and/or PE- conjugated intracellular antibody or the corresponding isotype control.
8. Vortex 1-2 seconds and incubate for 20 minutes.
9. Wash once in 3 ml PBS + 0.1% NaN_3 + 5% FBS.
10. Centrifuge for 5 minutes at 300-350 x g and aspirate the supernatant.
11. Resuspend cells in sheath fluid for immediate analysis or in 0.5 ml of 0.1% paraformaldehyde fixative solution for storage at 2-8°C in the dark. Fixed cells should be analyzed within 24 hours.

METHANOL MODIFICATION

1. For each sample to be analyzed add the appropriate volume of adjusted cells (equivalent to 1×10^6 cells) to a 5 ml, 12x75 mm tube.
2. Add 100 μ l of Reagent A (Fixation Medium) to each tube and incubate for 2-3 minutes at room temperature.
3. Add 2-4 ml of precooled absolute methanol (0-4°C), vortex and incubate for an additional 10 minutes at 0-4°C.
4. Centrifuge for 5 minutes at 300-350 x g and wash once with 3 ml of PBS + 0.1% NaN_3 + 5% FBS.
5. Centrifuge for 5 minutes at 300-350 x g, aspirate the supernatant, and vortex to fully resuspend the cell pellet.
6. Add 100 μ l of Reagent B (Permeabilization Medium) and the recommended volume of the FITC-conjugated intracellular antibody or the corresponding isotype control.
8. Vortex 1-2 seconds and incubate for 30 minutes at room temperature.
9. Wash once in 3 ml PBS + 0.1% NaN_3 + 5% FBS.
10. Centrifuge for 5 minutes at 300-350 x g.
11. Resuspend cells in sheath fluid for immediate analysis or in 0.5 ml of 0.1% paraformaldehyde fixative solution for storage at 2-8°C in the dark. Fixed cells should be analyzed within 24 hours.

The methanol modification is recommended for cell cycle antigens such as Ki-67, BrdU and PCNA when using FITC conjugated antibodies. It is not recommended when using R-PE conjugated antibodies.

- Notes:**
- a. For intracellular Ig detection, double washes should be used to remove soluble Ig.
 - b. In some cases, the pellet may be difficult to resuspend after a 5 minutes centrifugation... reduce to 1-2 minutes to solve this problem.

Applications Using FIX & PERM®

APPLICATIONS USING FIX & PERM®

AN-DER-GRUB Bioresearch (Austria) has pioneered the development of applications for intracellular antigen detection by flow cytometry. FIX & PERM®, the fixation and permeabilization reagent system developed and manufactured by AN-DER-GRUB and marketed by CALTAG Laboratories, has effectively taken the guesswork out of intracellular flow cytometry. Since the introduction of this unique reagent system, the number of proven intracellular staining applications has grown rapidly. Among the applications identified to date are the following:

LYSOSOMAL PROTEINS

Elastase

Lactoferrin

Lysozyme

Myeloperoxidase

Proteinase-3

CYTOPLASMIC CD MOLECULES

CD3

CD13

CD22

CD62P

CD63

CD68

CD79a

NUCLEAR PROLIFERATION MARKERS

BrdU

Ki-67

PCNA

NUCLEAR ENZYMES

TdT

ONCOPROTEINS

Bcl-2

c-myc

p53

HIV ANTIGENS

p24

CYTOKINES/CHEMOKINES

Human: GM-CSF

IFN- γ

TNF- α

IL1- β

IL-2

IL-4

IL-10

Rantes

Murine: IFN- α

IFN- γ

TNF- α

IL-2

IL-4

IL-5

IL-10

IMMUNOGLOBULINS

Human: IgA

IgG

IgD

IgM

κ

λ

Murine: IgG

IgM

OTHER MOLECULES

MHC Class II

Phosphotyrosine

Thrombospondin

CMV Ag.

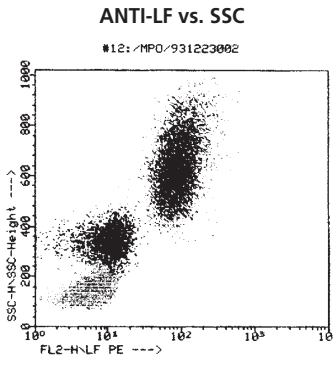
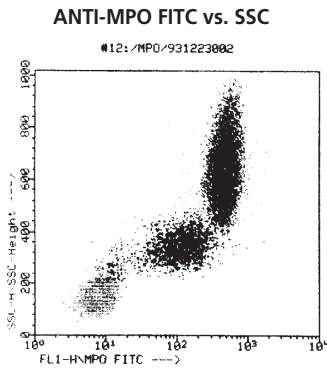
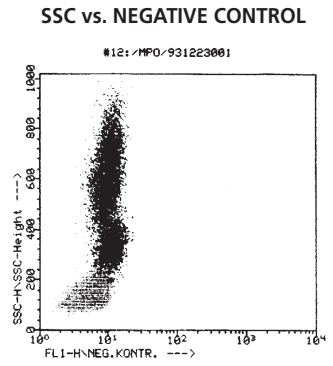
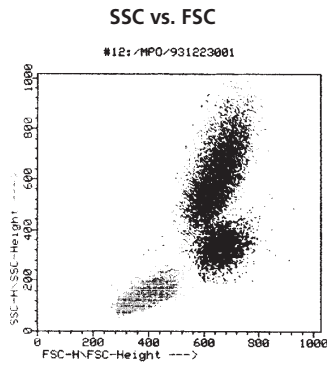
Cyclins

Transfected Cells

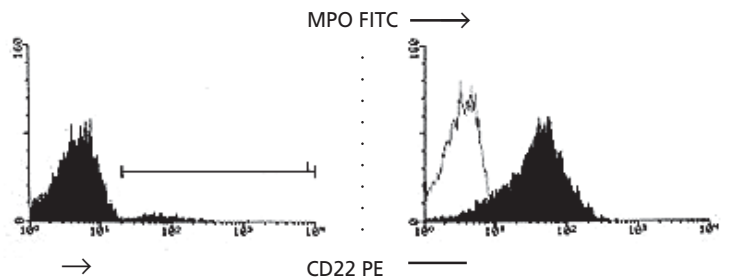
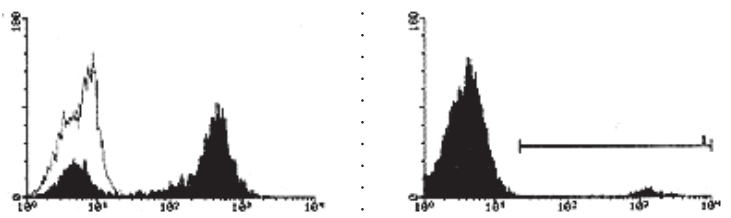
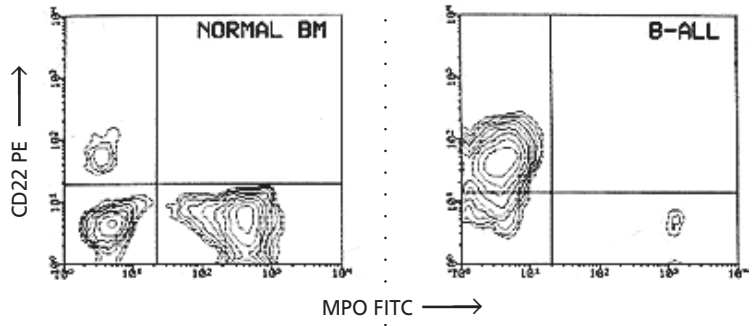
MDR (Multi-Drug Resistant)

Typical Flow Cytometric Patterns

SCATTER PATTERNS

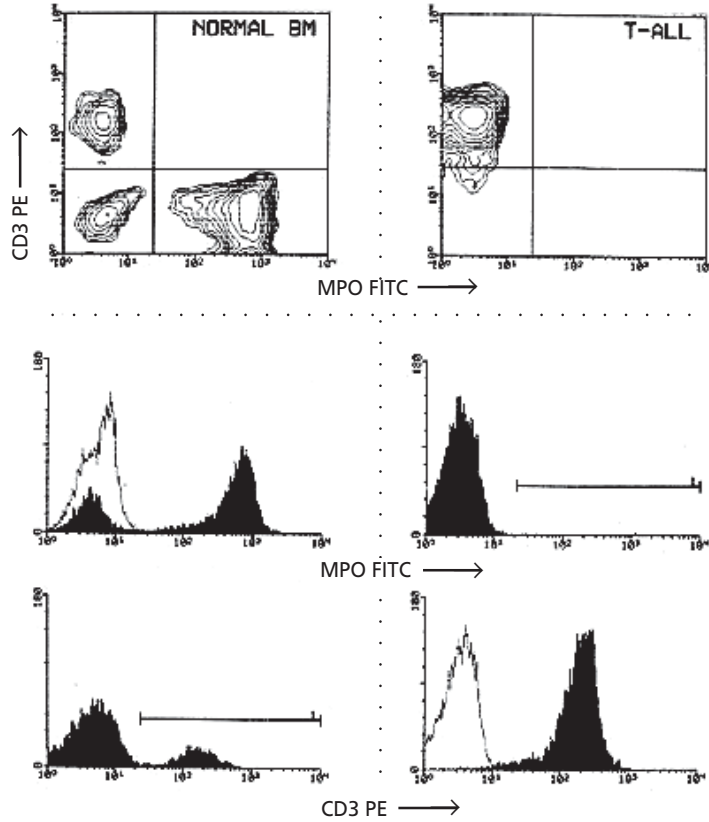


CYTOPLASMIC MYELOPEROXIDASE/CD22 NORMAL BONE MARROW vs. B-ALL

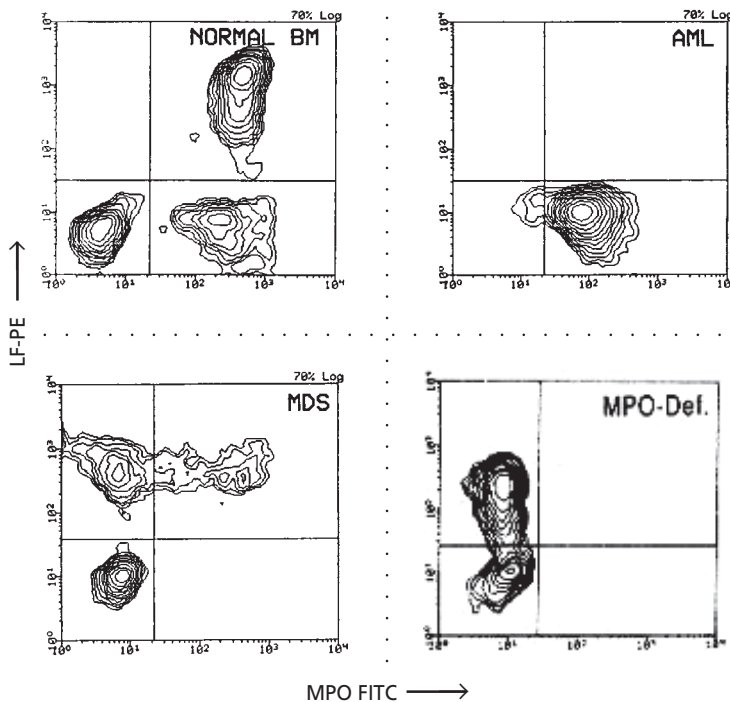


**CYTOPLASMIC MYELOPEROXIDASE/CD3
NORMAL BONE MARROW vs. T-ALL**

Typical Flow Cytometric Patterns

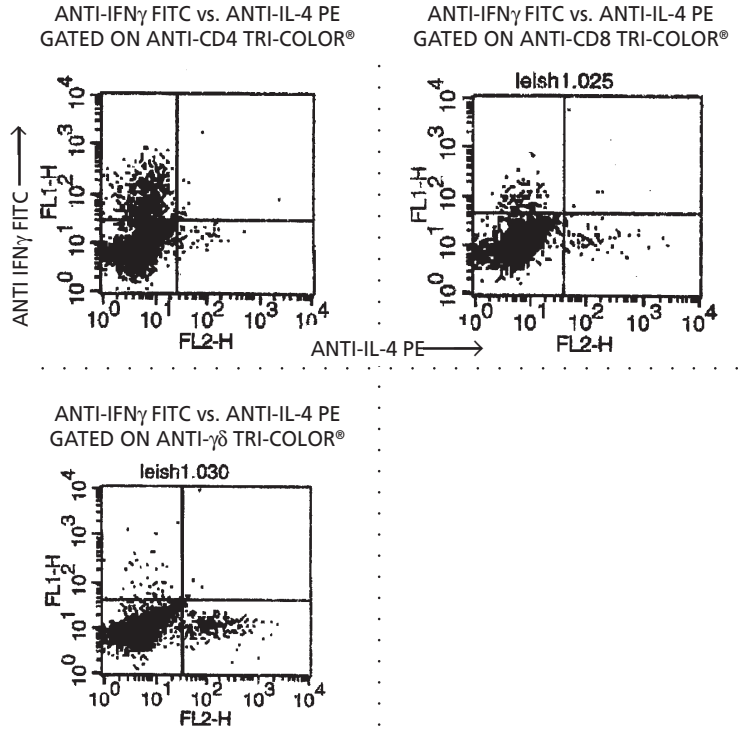


FLOW CYTOMETRIC ANALYSIS OF INTRACELLULAR MYELOPEROXIDASE AND LACTOFERRIN—AML, MDS, MPO DEFICIENCY



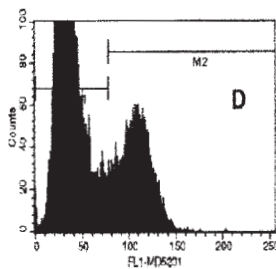
Typical Flow Cytometric Patterns

INTRACELLULAR CYTOKINE DETECTION BY FLOW CYTOMETRY SPLENOCYTES FROM LEISHMANIA-INFECTED MICE

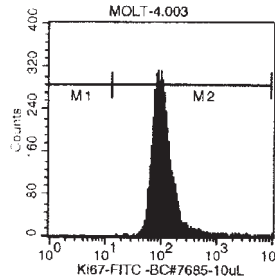


CELL CYCLE ANTIGENS USING METHANOL MODIFICATION

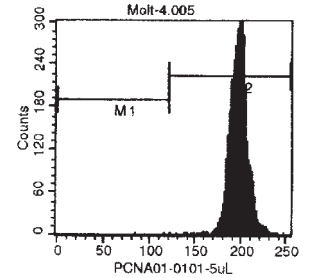
ANTI-BrdU
BIO-E FLUOROTROL $^{\text{TM}}$ DNA PLUS
(PERMEABILIZED W. REAGENT B)



ANTI-Ki-67 FITC
MOLT 4 CELLS

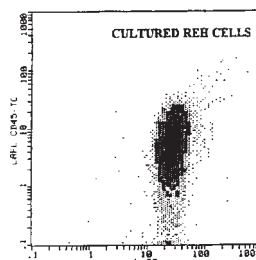


ANTI-PCNA FITC
MOLT 4 CELLS



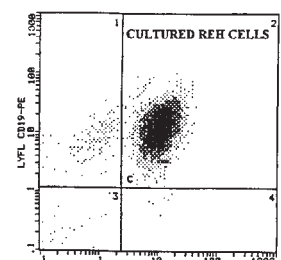
THREE COLOR STAINING FOR SURFACE CD19 AND INTRACELLULAR TdT USING CD45 TC VS. SIDE SCATTER GATING

ANTI-CD45 TRI-COLOR $^{\circ}$ vs. SSC



CD45T vs. Side Scatter

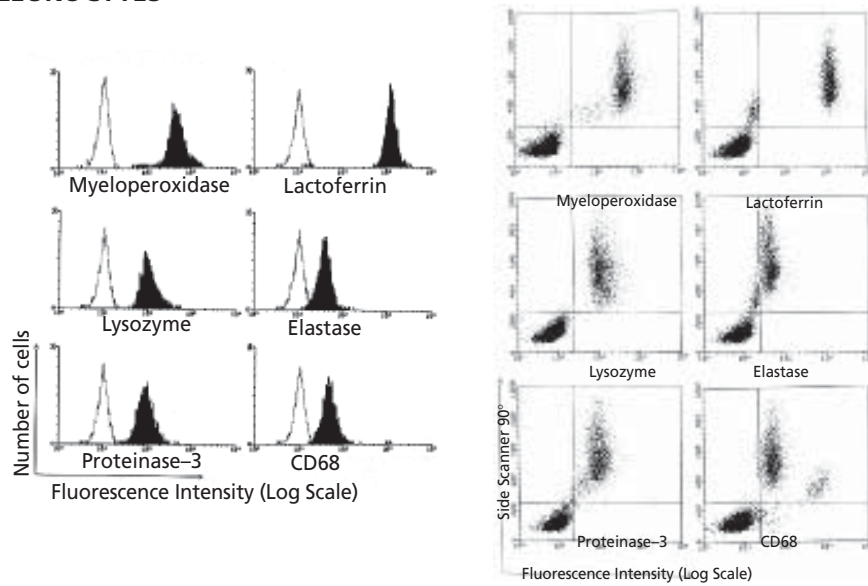
ANTI-CD19PE vs. ANTI-Tdt FITC



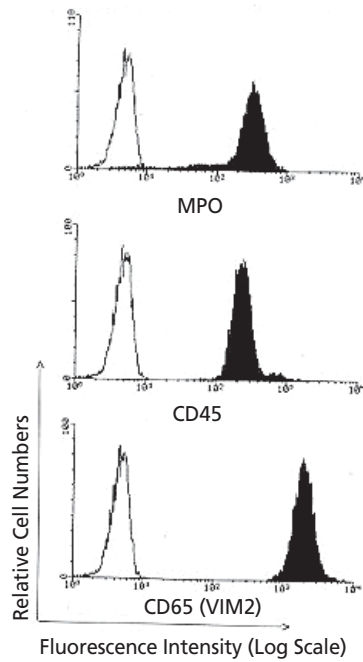
CD19 PE vs. Tdt FITC

FLOW CYTOMETRIC ANALYSIS OF LYSOSOMAL ANTIGENS IN PERIPHERAL BLOOD LEUKOCYTES

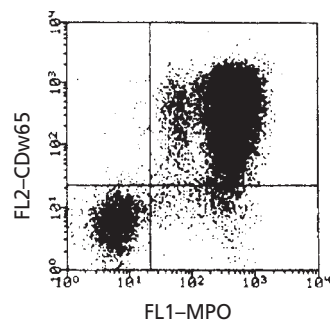
Typical Flow Cytometric Patterns



FLUORESCENCE INTENSITY OF INTRACELLULAR MPO VS. SURFACE CD45 AND CD65 ON HUMAN PB GRANULOCYTES



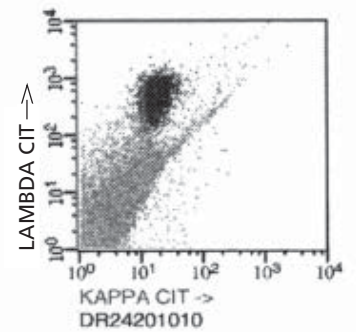
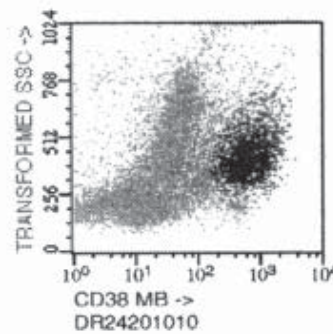
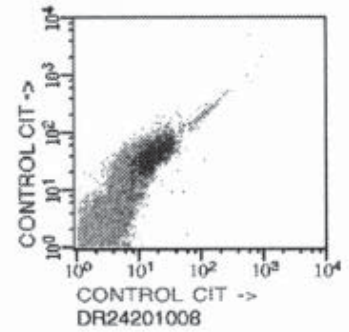
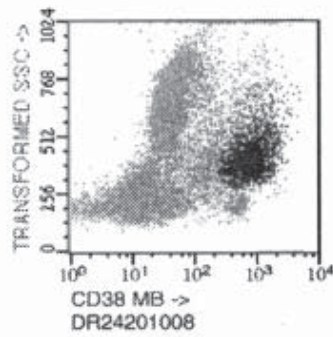
ANTI-CD65 PE (SURFACE) VS. ANTI-MPO FITC (INTRACELLULAR)



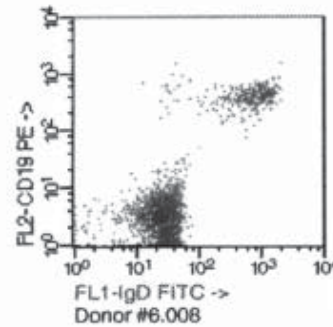
Typical Flow Cytometric Patterns

INTRACELLULAR Ig DETECTION

Kappa/Lambda



IgD - Gated on CD19⁺ B-cells

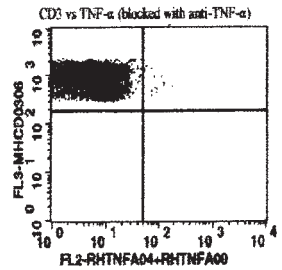
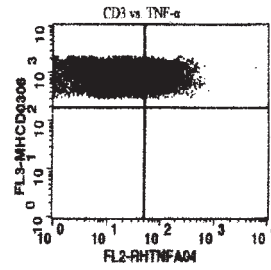
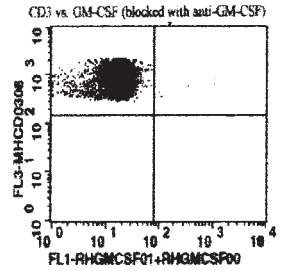
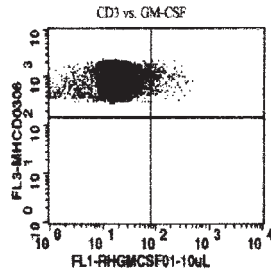
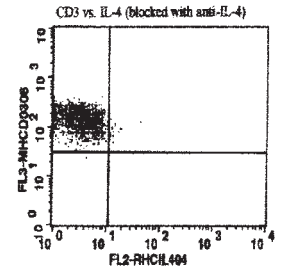
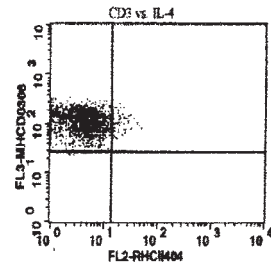
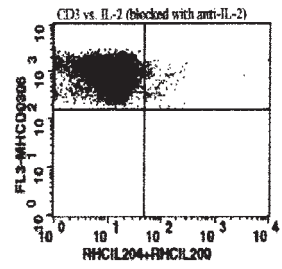
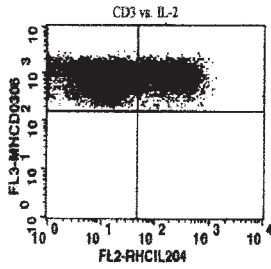
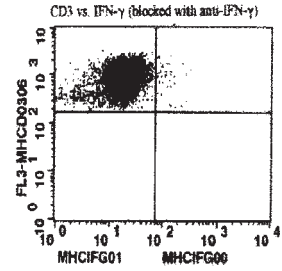
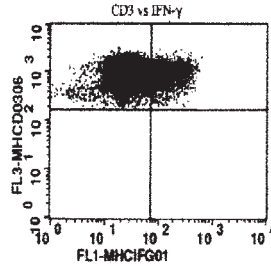


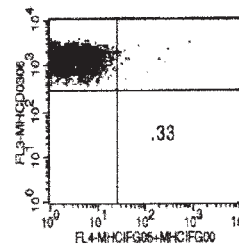
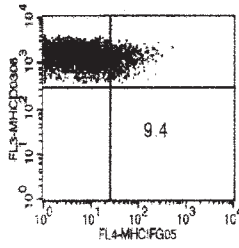
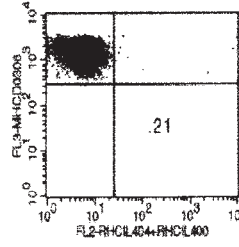
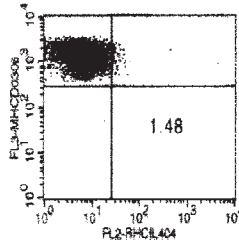
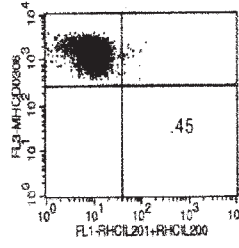
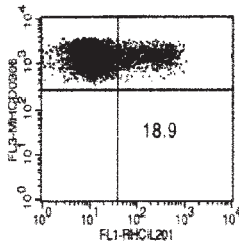
VERY IMPORTANT: blood must be collected into heparinized tubes

1. Aliquot 0.5 ml heparinized, whole blood into each of two 12x75 mm snap cap tubes. Add 0.5 ml RPMI 1640 w/o additives to each tube to bring volume to 1ml.
2. To the first tube add 10 µg Brefeldin A (20 µl of a stock solution of Brefeldin A at a concentration of 0.5 mg/ml in 200 proof EtOH and stored at -20°C). Mix contents of tube gently. This tube is the resting cell population.
3. To the second tube also add 10 µg Brefeldin A, 25 ng PMA (2.5 µl of a stock solution of PMA made up at a concentration of 1 mg/ml in DMSO and diluted 1:100 in RPMI 1640 w/o additives and stored at -20°C) and 1 µg ionomycin (1µl of a stock solution of ionomycin at a concentration of 1mg/ml in DMSO and stored at -20°C). Mix contents of tube gently. This is the activated cell population.
4. Incubate for 4 hrs. at 37°C in 7.5% humidified CO₂ incubator.
5. At the end of the incubation period mix cells again and aliquot 100 µl into 12x75 mm snap cap tubes.
6. Add 5 µl of appropriate Caltag antibody for phenotyping cells (cell surface staining).....e.g. CD3-TRI-COLOR®(TC), CD4-TC, CD8-TC, CD45-TC or CD69-TC for a three color staining assay. Either FITC or R-PE may be appropriate for a two color assay.
7. Incubate in the dark for 15 minutes at room temperature.
8. Add 100 µl of Reagent A from the FIX & PERM kit. Mix cells gently and incubate for an additional 15 minutes at room temperature in the dark.
9. Wash twice with wash medium (PBS+1%BSA, 0.1% NaN₃, 1% FBS). The pellet becomes less cohesive on the second wash so use caution to avoid losing cells.
10. To the washed cells add 100 µl of Reagent B from the FIX & PERM kit and 1-10 µl of each anti-cytokine antibody OR of isotype control.
11. Incubate for 20 minutes at room temperature.
12. Wash twice with wash medium and resuspend pellet in FACS fix.
13. Analyze by gating on SSC and FL-3 (3 or 4 color assay).

Typical Flow Cytometric Patterns

CYTOKINE DETECTION IN HUMAN WHOLE BLOOD (3 Color Assay - Gated on CD3 TRI-COLOR®)





Reagents Available From CALTAG

FIX & PERM® CELL PERMEABILIZATION REAGENTS

| | VOLUME | TESTS | CODE |
|--|------------------------------------|-------|-------------|
| Fixation and Permeabilization Kit | 1 x 5 ml Fixation Medium | 50 | GAS-003 |
| | 1 x 5 ml Permeabilization Medium | | |
| Fixation and Permeabilization Kit | 4 x 5 ml Fixation Medium | 200 | GAS-004 |
| | 4 x 5 ml Permeabilization Medium | | |
| Fixation Medium-Bulk | 1 x 5 ml Fixation Medium | 50 | GAS001 |
| | 1 x 100 ml Fixation Medium | 1000 | GAS001S-100 |
| Permeabilization Medium-Bulk | 1 x 5 ml Permeabilization Medium | 50 | GAS002 |
| | 1 x 100 ml Permeabilization Medium | 1000 | GAS002S-100 |

COMBI - IC® MONOCLONAL ANTIBODIES

For Intracellular Antigen Detection

The following COMBI-IC® monoclonal antibodies have been optimized for use with the FIX & PERM® cell permeabilization kits. Single conjugates, as well as premixed, FITC/R-PE combinations are available for the detection of human myeloid, T-cell and B-cell antigens by flow cytometry.

| ANTIBODY/CLONE | CODE | FORM | VOL. | TESTS |
|---|-------------|---------|--------|---------|
| Myeloperoxidase (MPO) & Lactoferrin (LF) | GIC 202-25 | 2 color | 0.5 ml | 25 min |
| Anti-Myeloperoxidase FITC (H-43-5) Mouse IgG1 | GIC 202-100 | 2 color | 2.0 ml | 100 min |
| Anti-Lactoferrin R-PE (3C5) Mouse IgG1 | | | | |
| Myeloperoxidase (MPO) & CD3 | GIC 203-25 | 2 color | 0.5 ml | 25 min |
| Anti-Myeloperoxidase FITC (H-43-5) Mouse IgG1 | GIC 203-100 | 2 color | 2.0 ml | 100 min |
| Anti-CD3 R-PE (UCHT1) Mouse IgG1 | | | | |
| Myeloperoxidase (MPO) & CD22 | GIC 204-25 | 2 color | 0.5 ml | 25 min |
| Anti-MPO FITC (H-43-5) Mouse IgG1 | GIC 204-100 | 2 color | 2.0 ml | 100 min |
| Anti-CD22 R-PE (RFB4) Mouse IgG1 | | | | |
| Negative Control | GIC 201-50 | 2 color | 1.0 ml | 50 min |
| Mouse IgG1 FITC / Mouse IgG1 R-PE | | | | |
| Anti-MPO | GIC 205 | FITC | 1.0 ml | 50 min |
| Anti-LF | GIC 206 | R-PE | 1.0 ml | 50 min |
| Anti-Lysozyme | GIC207 | FITC | 1.0 ml | 50 min |
| Clone LZ-1 Mouse IgG1 | | | | |
| CD68 | GM4152 | FITC | 1.0 ml | 50 min |
| Clone KIM7 Mouse IgG1 | | | | |
| CD3 | GM4013-5 | R-PE | 1.0 ml | 50 min |
| Clone UCHT1 Mouse IgG1 | | | | |
| TdT | MHTDT01-2 | FITC | 0.2 ml | 20 min |
| Clone TdT-6 Mouse IgG1 | MHTDT01-5 | FITC | 0.5 ml | 50 min |

CYTO-IC® MONOCLONAL ANTIBODIES

For Intracellular Murine Cytokine Detection

| ANTIBODY/CLONE | CODE | FORM | VOL. | ANTIBODY | TESTS |
|---|------------|-----------|-------------|-------------|---------|
| Rat Anti-Mouse IFN-α | RM9034 | R-PE | 0.5 ml | 50 μ g | 50 min |
| Clone HM1001 | RM9034-3 | R-PE | 3.0 ml | 300 μ g | 300 min |
| Rat IgG1 | HBT-HM1001 | Pur. Ab. | Lyophilized | 200 μ g | |
| Rat Anti-Mouse IFN-γ | RM9000 | Pur. Ab. | 1.0 ml | 200 μ g | |
| Clone XMG1.2 | RM90020 | Alexa 488 | 1.0 ml | 100 μ g | 100 min |
| Rat IgG1 | RM9001 | FITC | 1.0 ml | 100 μ g | 100 min |
| | RM9001-3 | FITC | 3.0 ml | 300 μ g | 300 min |
| | RM9004 | R-PE | 0.5 ml | 50 μ g | 50 min |
| | RM9004-3 | R-PE | 3.0 ml | 300 μ g | 300 min |
| Rat Anti-Mouse IL-2 | RM9020 | Pur. Ab. | 1.0 ml | 200 μ g | |
| Clone JES6-5H4 | RM9021 | FITC | 1.0 ml | 100 μ g | 100 min |
| Rat IgG2b | RM9021-3 | FITC | 3.0 ml | 300 μ g | 300 min |
| | RM9024 | R-PE | 0.5 ml | 50 μ g | 50 min |
| | RM9024-3 | R-PE | 3.0 ml | 300 μ g | 300 min |
| Rat Anti-Mouse IL-4 | RM9050 | Pur. Ab. | 1.0 ml | 200 μ g | |
| Clone BVD-24G2 | RM90520 | Alexa 488 | 1.0 ml | 100 μ g | 100 min |
| Rat IgG1 | RM9051 | FITC | 1.0 ml | 100 μ g | 100 min |
| | RM9051-3 | FITC | 3.0 ml | 300 μ g | 300 min |
| | RM9054 | R-PE | 0.5 ml | 50 μ g | 50 min |
| | RM9054-3 | R-PE | 3.0 ml | 300 μ g | 300 min |
| Rat Anti-Mouse IL-5 | RM9060 | Pur. Ab. | 1.0 ml | 200 μ g | |
| Clone TRFK5 | RM9061 | FITC | 1.0 ml | 100 μ g | 100 min |
| Rat IgG1 | RM9061-3 | FITC | 3.0 ml | 300 μ g | 300 min |
| | RM9064 | R-PE | 0.5 ml | 50 μ g | 50 min |
| | RM9064-3 | R-PE | 3.0 ml | 300 μ g | 300 min |
| Rat Anti-Mouse IL-10 | RM9100 | Pur. Ab. | 1.0 ml | 200 μ g | |
| Clone JES5-2A5 | RM9101 | FITC | 1.0 ml | 100 μ g | 100 min |
| Rat IgG1 | RM9101-3 | FITC | 3.0 ml | 300 μ g | 300 min |
| | RM9104 | R-PE | 0.5 ml | 50 μ g | 50 min |
| | RM9104-3 | R-PE | 3.0 ml | 300 μ g | 300 min |
| Rat Anti-Mouse TNF-α | RM9010 | Pur. Ab. | 1.0 ml | 200 μ g | |
| Clone MP6-XT22 | RM9011 | FITC | 1.0 ml | 100 μ g | 100 min |
| Rat IgG1 | RM9011-3 | FITC | 3.0 ml | 300 μ g | 300 min |
| | RM9014 | R-PE | 0.5 ml | 50 μ g | 50 min |
| | RM9014-3 | R-PE | 3.0 ml | 300 μ g | 300 min |

Reagents
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CALTAG

Reagents Available From CALTAG

CYTO-IC® MONOCLONAL ANTIBODIES

For Intracellular Human Cytokine Detection

| ANTIBODY/CLONE | CODE | FORM | VOL. | ANTIBODY | TESTS |
|---|--------------|-----------|--------|-------------|---------|
| Mouse Anti-Human IFN-γ | MHCIFG00 | Pur. Ab. | 1.0 ml | 200 μ g | |
| Clone B27 | MHCIFG20 | Alexa 488 | 0.5 ml | | 100 min |
| Mouse IgG1 | MHCIFG01 | FITC | 1.0 ml | | 100 min |
| | MHCIFG01-3 | FITC | 3.0 ml | | 300 min |
| | MHCIFG04 | R-PE | 0.5 ml | | 50 min |
| | MHCIFG04-3 | R-PE | 3.0 ml | | 300 min |
| | MHCIFG05 | APC | 0.5 ml | | 100 min |
| Rat Anti-Human IL-2 | RHCIL200 | Pur. Ab. | 1.0 ml | 200 μ g | |
| Clone MQ1-17H12 | RHCIL201 | FITC | 1.0 ml | | 100 min |
| Rat IgG2a | RHCIL201-3 | FITC | 3.0 ml | | 300 min |
| | RHCIL204 | R-PE | 0.5 ml | | 50 min |
| | RHCIL204-3 | R-PE | 3.0 ml | | 300 min |
| | RHCIL205 | APC | 0.5 ml | | 100 min |
| Rat Anti-Human IL-4 | RHCIL400 | Pur. Ab. | 1.0 ml | 200 μ g | |
| Clone MP4-25D2 | RHCIL420 | Alexa 488 | 0.5 ml | | 100 min |
| Rat IgG1 | RHCIL401 | FITC | 1.0 ml | | 100 min |
| | RHCIL401-3 | FITC | 3.0 ml | | 300 min |
| | RHCIL404 | R-PE | 0.5 ml | | 50 min |
| | RHCIL404-3 | R-PE | 3.0 ml | | 300 min |
| Rat Anti-Human IL-10 | RHCIL1000 | Pur. Ab. | 1.0 ml | 200 μ g | |
| Clone JES3-9D7 | RHCIL1001 | FITC | 1.0 ml | | 100 min |
| Rat IgG1 | RHCIL1001-3 | FITC | 3.0 ml | | 300 min |
| | RHCIL1004 | R-PE | 0.5 ml | | 50 min |
| | RHCIL1004-3 | R-PE | 3.0 ml | | 300 min |
| Mouse Anti-Human IL-1β | MHCIL1B01 | FITC | 1.0 ml | | 50 min |
| Clone IL1B | | | | | |
| Rat Anti-Human TNF-α | RHCNFA00 | Pur. Ab. | 1.0 ml | 200 μ g | |
| Clone MP9-20A4 | RHCNFA01 | FITC | 1.0 ml | | 100 min |
| Rat IgG1 | RHCNFA01-3 | FITC | 3.0 ml | | 300 min |
| | RHCNFA04 | R-PE | 0.5 ml | | 50 min |
| | RHCNFA04-3 | R-PE | 3.0 ml | | 300 min |
| | RHCNFA05 | APC | 0.5 ml | | 100 min |
| Rat Anti-Human GM-CSF | RHGMCSF00 | Pur. Ab. | 1.0 ml | 200 μ g | |
| Clone BVD2-21C11 | RHGMCSF01 | FITC | 1.0 ml | | 100 min |
| Rat IgG2a | RRHGMCSF01-3 | FITC | 3.0 ml | | 300 min |
| | RHGMCSF04 | R-PE | 0.5 ml | | 50 min |
| | RHGMCSF04-3 | R-PE | 3.0 ml | | 300 min |
| Mouse Anti-Human Rantes VL1 | RANT100 | Pur. Ab. | 1.0 ml | 100 μ g | |
| Clone VL1 | RANT101 | FITC | 1.0 ml | | 100 min |
| Rat IgG2b | RANT104 | R-PE | 1.0 ml | | 100 min |

ADDITIONAL CALTAG ANTIBODIES

For Intracellular Antigen Detection

| ANTIBODY/CLONE | CODE | FORM | VOL. | TESTS |
|---|---------------------------------|---------------------------|----------------------------|-------------------------------|
| Mouse Anti-BrdU Clone 3D4 Mouse IgG1 | MD5401 | FITC | 0.5 ml | 100 min |
| Mouse Anti-Human Bcl-2 Clone 100 Mouse IgG1 | MHBCL01 MHBCL01-4 MHBCL04 | FITC FITC R-PE | 0.5 ml 2.0 ml 0.5 ml | 100 min 400 min 100 min |
| Mouse Anti-Human PCNA Clone PC10 Mouse IgG2a | PCNA01 PCNA04 | FITC R-PE | 0.5 ml 0.5 ml | 100 min 100 min |
| Mouse Anti-Human κ Clone HP6062 Mouse IgG3 | MH10520 MH10511 MH10514 | Alexa 488 FITC R-PE | 0.5 ml 0.5 ml 0.5 ml | 100 min 100 min 100 min |
| Mouse Anti-Human λ Clone HP6054 Mouse IgG2a | MH10620 MH10611 MH10614 | Alexa 488 FITC R-PE | 0.5 ml 0.5 ml 0.5 ml | 100 min 100 min 100 min |
| Goat F(ab')₂ Anti-Human κ | H16101 H16104 | FITC R-PE | 1.0 ml 0.5 ml | 700 min 100 min |
| Goat F(ab')₂ Anti-Human λ | H16601 H16604 | FITC R-PE | 1.0 ml 0.5 ml | 700 min 100 min |
| Goat F(ab')₂ Anti-Human IgG (γ chain) | H10101 H10104 | FITC R-PE | 1.0 ml 0.5 ml | 700 min 100 min |
| Goat F(ab')₂ Anti-Human IgM (μ chain) | H15101 H15104 | FITC R-PE | 1.0 ml 0.5 ml | 700 min 100 min |
| Goat F(ab')₂ Anti-Human IgD (δ chain) | H15501 | FITC | 1.0 ml | 700 min |
| Goat F(ab')₂ Anti-Human IgA (α chain) | H14101 | FITC | 1.0 ml | 700 min |

OTHER USEFUL ANTIBODIES FOR INTRACELLULAR FLOW CYTOMETRY

ANTIBODY

CD79a Under Development

ANTIBODY

Anti-p24 Available From Beckman Coulter

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CALTAG

LABORATORIES

1849 Bayshore Blvd., #200
Burlingame, CA 94010
Tel: 800/874-4007
Fax: 650/652-9030
E-mail: caltag@caltag.com
Web Site: www.caltag.com

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