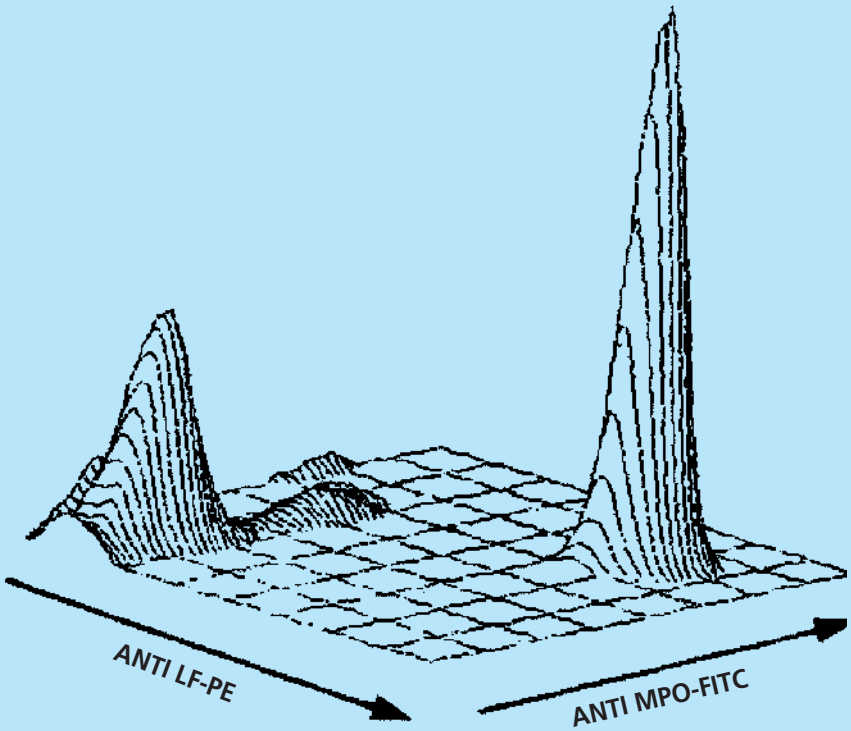
FOURTH EDITION

Additional References

Protocols for Cytokines in Human Whole Blood

FIX & PERM®

Applications Guide to Intracellular Flow Cytometry



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.

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Contents

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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.

Use of FIX & PERM® Reagents

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 x 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 µl 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 µl 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 2-8ºC 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 x 10^6 cells) to a 5 ml, 12x75 mm tube.

2. Add 100 µl 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 (0-4ºC), vortex and incubate for an additional 10 minutes at 0-4ºC.

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 µl 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 2-8ºC 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

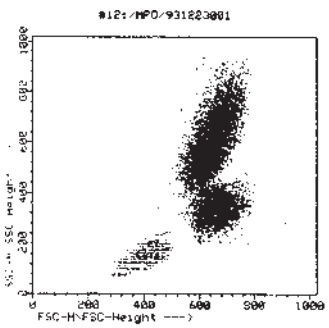
OTHER MOLECULES

- MHC Class II
- Phosphotyrosine
- Thrombospondin
- CMV Ag.
- Cyclins
- Transfected Cells
- MDR (Multi-Drug Resistant)

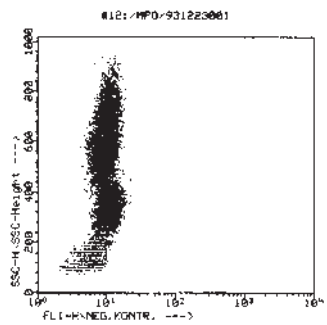
Typical Flow Cytometric Patterns

SCATTER PATTERNS

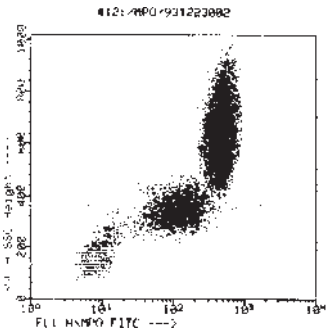
SSC vs. FSC



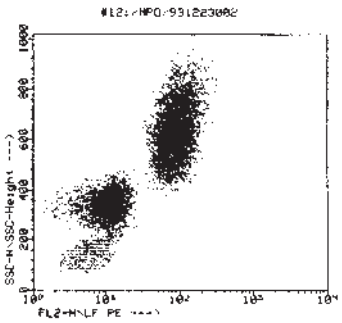
SSC vs. NEGATIVE CONTROL



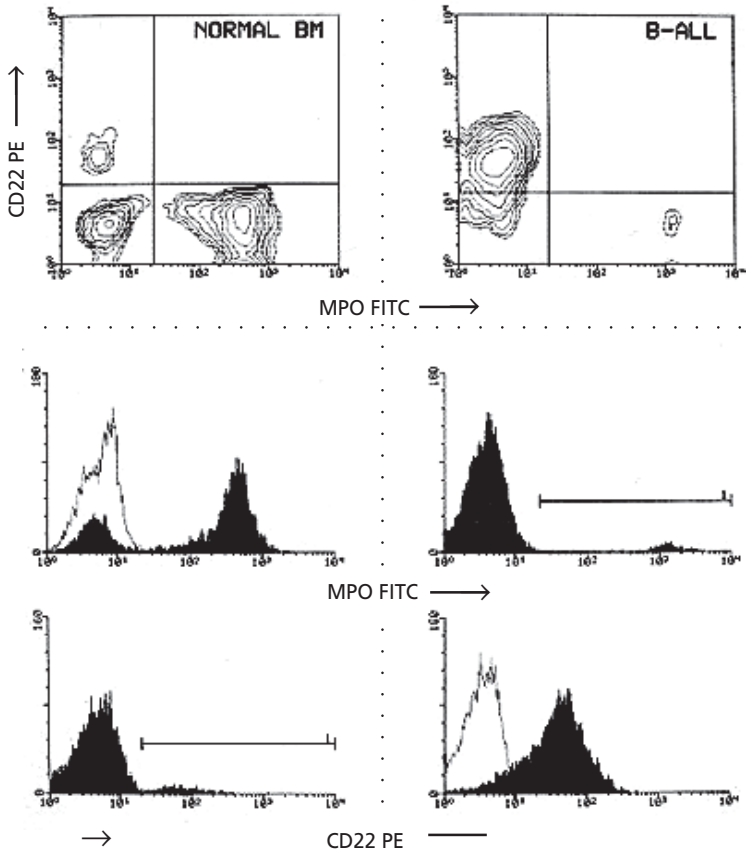
ANTI-MPO FITC vs. SSC



ANTI-LF vs. SSC

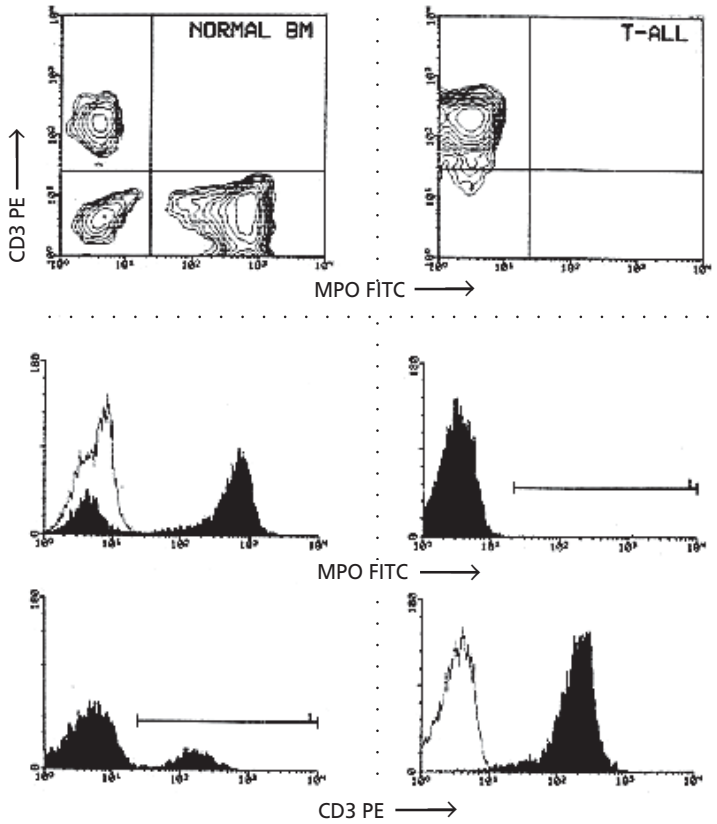


CYTOPLASMIC MYELOPEROXIDASE/CD22 NORMAL BONE MARROW vs. B-ALL

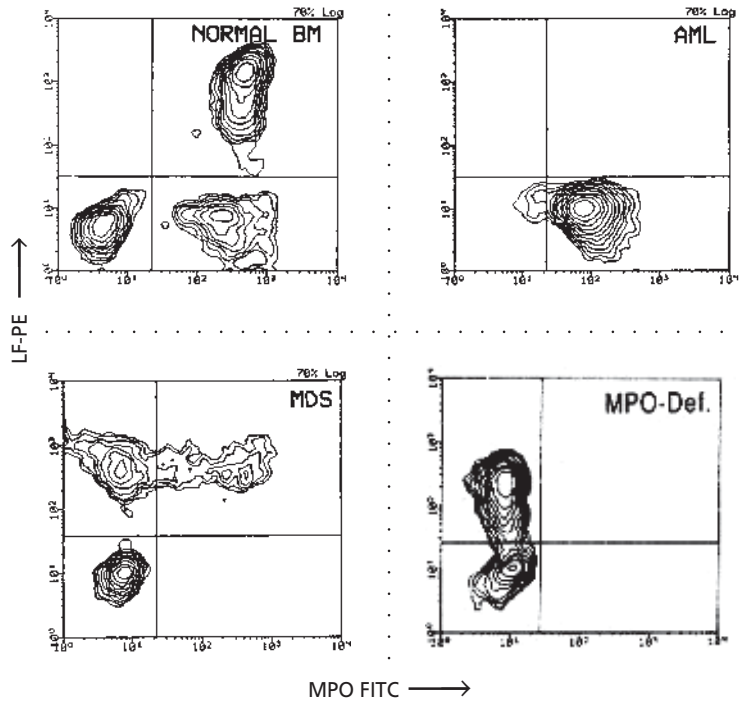


Typical Flow Cytometric Patterns

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



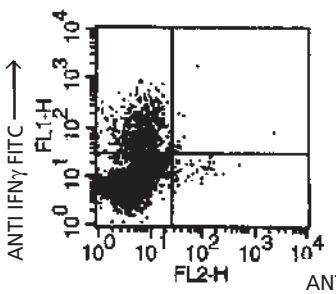
FLOW CYTOMETRIC ANALYSIS OF INTRACELLULAR MYELOPEROXIDASE AND LACTOFERRIN–AML, MDS, MPO DEFICIENCY



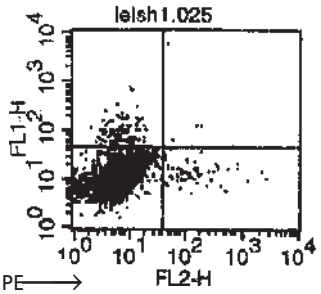
Typical Flow Cytometric Patterns

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

ANTI-IFNγ FITC vs. ANTI-IL-4 PE
GATED ON ANTI-CD4 TRI-COLOR®

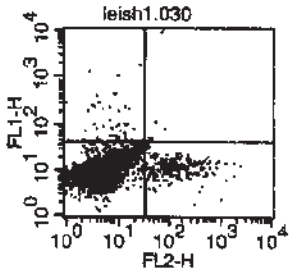


ANTI-IFNγ FITC vs. ANTI-IL-4 PE
GATED ON ANTI-CD8 TRI-COLOR®



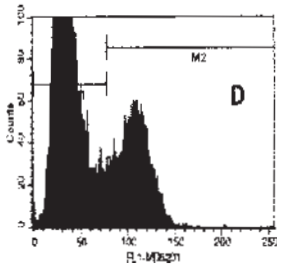
ANTI-IL-4 PE

ANTI-IFNγ FITC vs. ANTI-IL-4 PE
GATED ON ANTI-γδ TRI-COLOR®

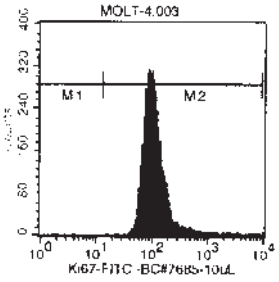


CELL CYCLE ANTIGENS USING METHANOL MODIFICATION

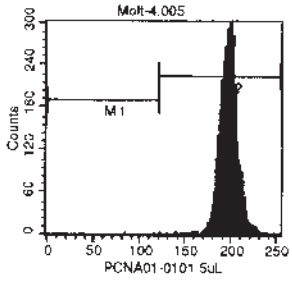
ANTI-BrdU
BIO-E FLUOROTROL™ DNA PLUS
(PERMEABILIZED W. REAGENT B)



ANTI-Ki-67 FITC
MOLT 4 CELLS

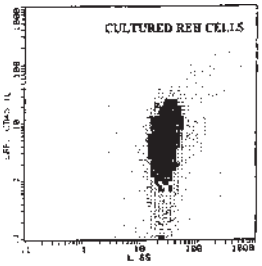


ANTI-PCNA FITC
MOLT 4 CELLS



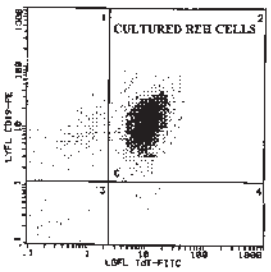
THREE COLOR STAINING FOR SURFACE CD19 AND INTRACELLULAR TDT USING CD45 TC VS. SIDE SCATTER GATING

ANTI-CD45 TRI-COLOR® vs. SSC



CD45T vs. Side Scatter

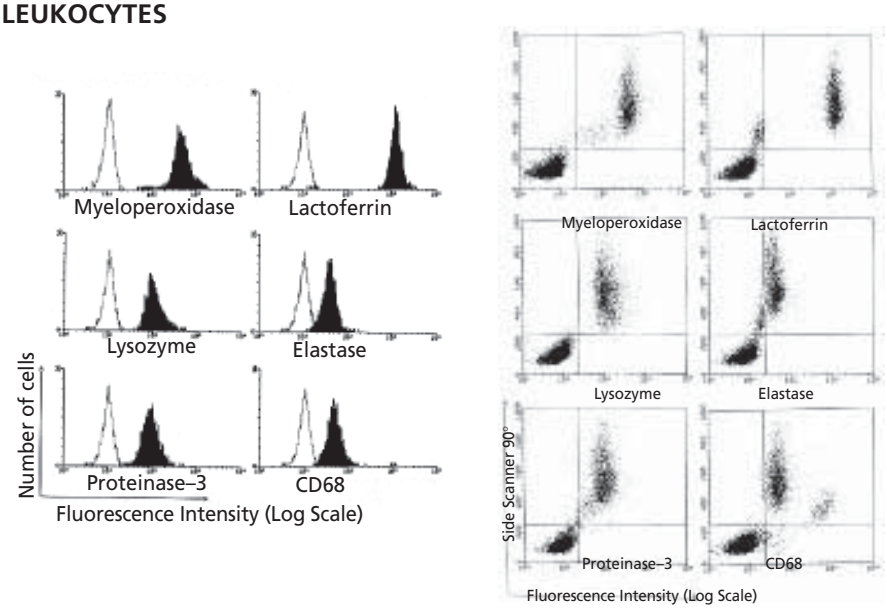
ANTI-CD19PE vs. ANTI-TdT FITC



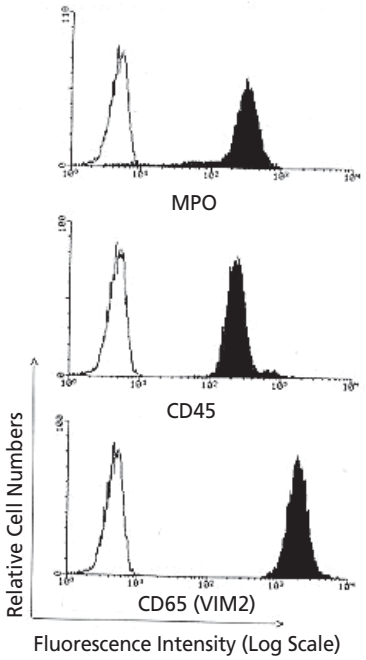
CD19 PE vs. TdT FITC

Typical Flow Cytometric Patterns

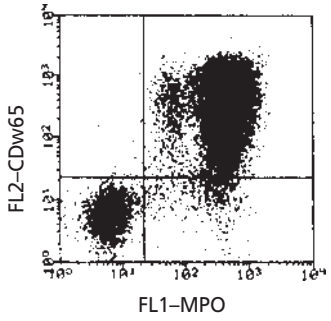
FLOW CYTOMETRIC ANALYSIS OF LYSOSOMAL ANTIGENS IN PERIPHERAL BLOOD LEUKOCYTES



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



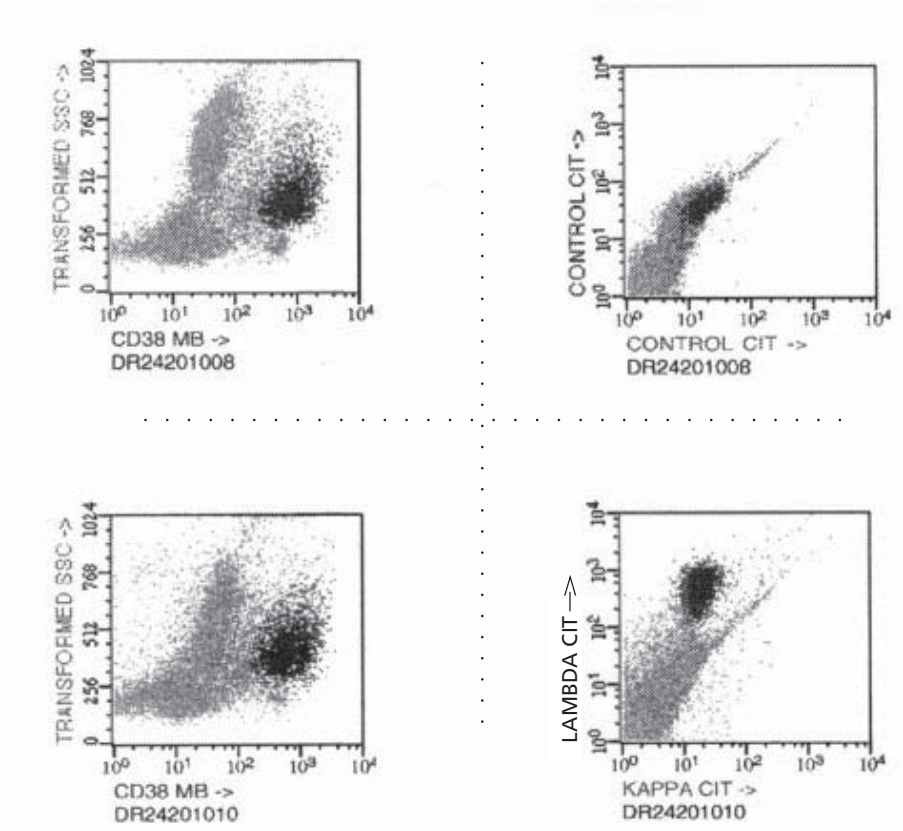
ANTI-CD65 PE (SURFACE) VS. ANTI-MPO FITC (INTRACELLULAR)



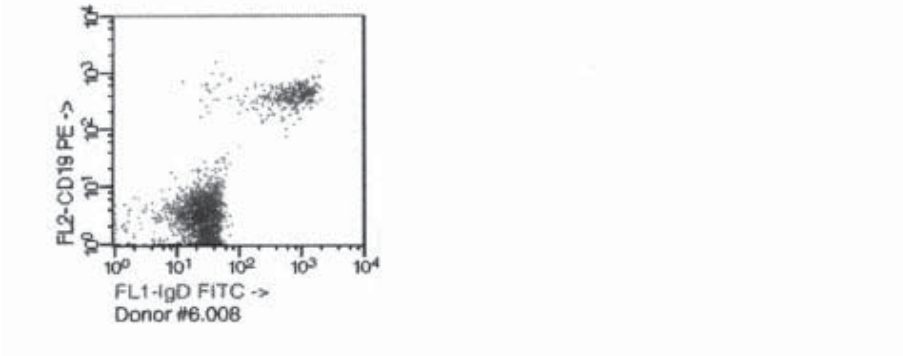
Typical Flow Cytometric Patterns

INTRACELLULAR Ig DETECTION

Kappa/Lambda



IgD - Gated on $CD19^+$ B-cells



PROTOCOL FOR STIMULATION OF HUMAN WHOLE BLOOD & INTRACELLULAR CYTOKINE STAINING WITH FIX & PERM®

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 CO_2 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 temperatue.

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% NaN_3, 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.

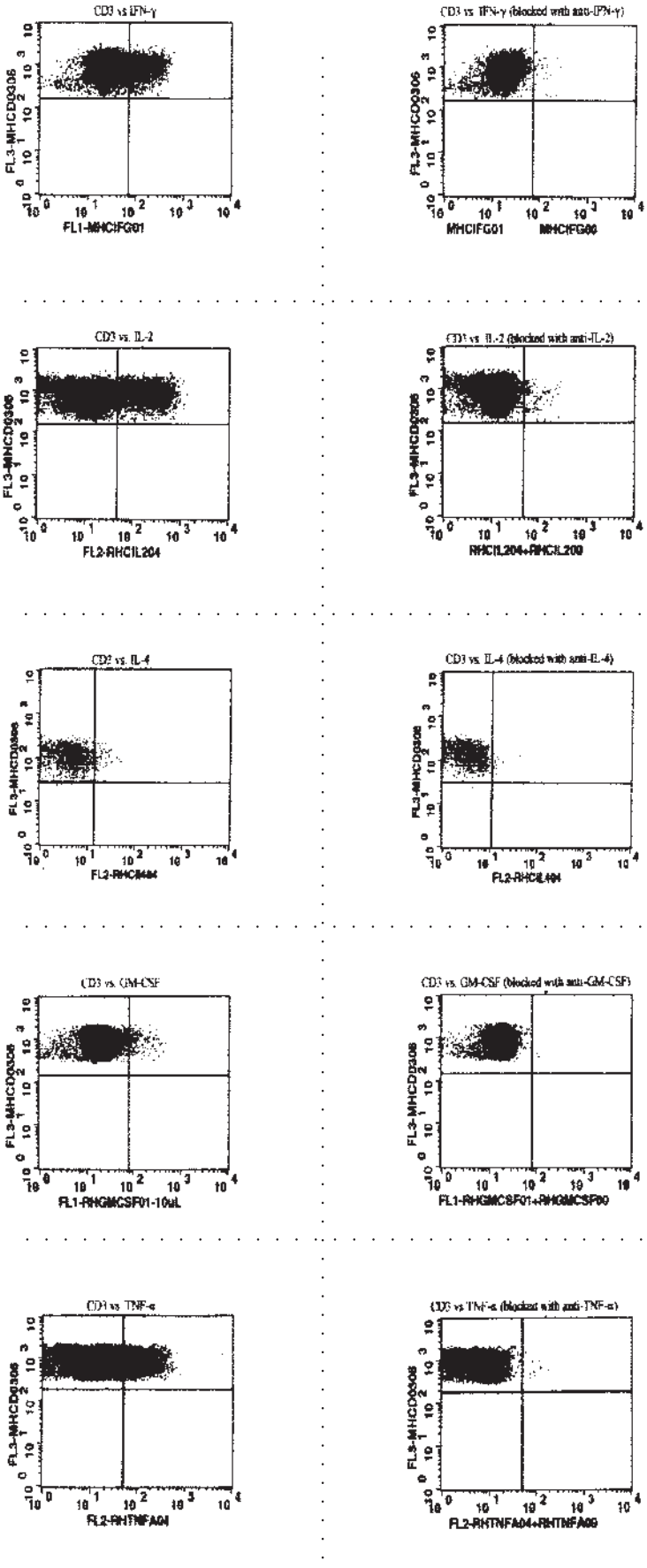
11. Incubate for 20 minutes at room temperature.

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).

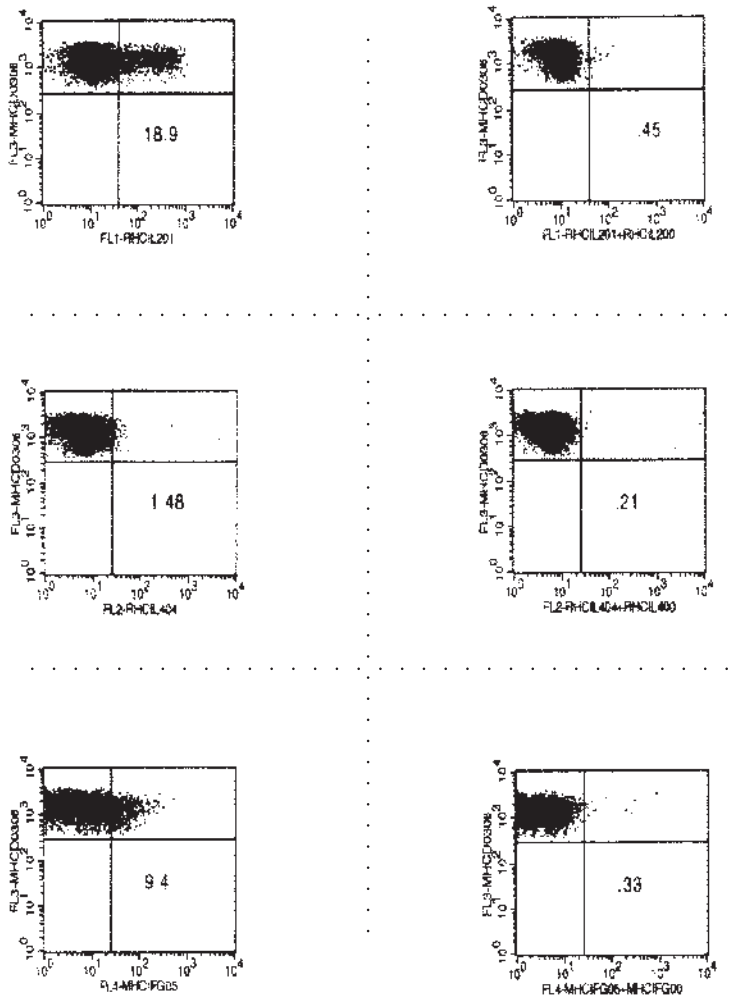
Typical Flow Cytometric Patterns

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



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

Typical Flow Cytometric Patterns



Reagents Available From CALTAG

FIX & PERM® CELL PERMEABILIZATION REAGENTS

	VOLUME	TESTS	CODE
Fixation and Permeabilization Kit	1 x 5 ml Fixation Medium 1 x 5 ml Permeabilization Medium	50	GAS-003
Fixation and Permeabilization Kit	4 x 5 ml Fixation Medium 4 x 5 ml Permeabilization Medium	200	GAS-004
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) Anti-Myeloperoxidase FITC (H-43-5) Mouse IgG1 Anti-Lactoferrin R-PE (3C5) Mouse IgG1	GIC 202-25 GIC 202-100	2 color 2 color	0.5 ml 2.0 ml	25 min 100 min
Myeloperoxidase (MPO) & CD3 Anti-Myeloperoxidase FITC (H-43-5) Mouse IgG1 Anti-CD3 R-PE (UCHT1) Mouse IgG1	GIC 203-25 GIC 203-100	2 color 2 color	0.5 ml 2.0 ml	25 min 100 min
Myeloperoxidase (MPO) & CD22 Anti-MPO FITC (H-43-5) Mouse IgG1 Anti-CD22 R-PE (RFB4) Mouse IgG1	GIC 204-25 GIC 204-100	2 color 2 color	0.5 ml 2.0 ml	25 min 100 min
Negative Control Mouse IgG1 FITC / Mouse IgG1 R-PE	GIC 201-50	2 color	1.0 ml	50 min
Anti-MPO	GIC 205	FITC	1.0 ml	50 min
Anti-LF	GIC 206	R-PE	1.0 ml	50 min
Anti-Lysozyme Clone LZ-1 Mouse IgG1	GIC207	FITC	1.0 ml	50 min
CD68 Clone KIM7 Mouse IgG1	GM4152	FITC	1.0 ml	50 min
CD3 Clone UCHT1 Mouse IgG1	GM4013-5	R-PE	1.0 ml	50 min
TdT Clone TdT-6 Mouse IgG1	MHTDT01-2 MHTDT01-5	FITC FITC	0.2 ml 0.5 ml	20 min 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 µg	50 min
Clone HM1001	RM9034-3	R-PE	3.0 ml	300 µg	300 min
Rat IgG1	HBT-HM1001	Pur. Ab.	Lyophilized	200 µg	
Rat Anti-Mouse IFN-γ	RM9000	Pur. Ab.	1.0 ml	200 µg	
Clone XMG1.2	RM90020	Alexa 488	1.0 ml	100 µg	100 min
Rat IgG1	RM9001	FITC	1.0 ml	100 µg	100 min
	RM9001-3	FITC	3.0 ml	300 µg	300 min
	RM9004	R-PE	0.5 ml	50 µg	50 min
	RM9004-3	R-PE	3.0 ml	300 µg	300 min
Rat Anti-Mouse IL-2	RM9020	Pur. Ab.	1.0 ml	200 µg	
Clone JES6-5H4	RM9021	FITC	1.0 ml	100 µg	100 min
Rat IgG2b	RM9021-3	FITC	3.0 ml	300 µg	300 min
	RM9024	R-PE	0.5 ml	50 µg	50 min
	RM9024-3	R-PE	3.0 ml	300 µg	300 min
Rat Anti-Mouse IL-4	RM9050	Pur. Ab.	1.0 ml	200 µg	
Clone BVD-24G2	RM90520	Alexa 488	1.0 ml	100 µg	100 min
Rat IgG1	RM9051	FITC	1.0 ml	100 µg	100 min
	RM9051-3	FITC	3.0 ml	300 µg	300 min
	RM9054	R-PE	0.5 ml	50 µg	50 min
	RM9054-3	R-PE	3.0 ml	300 µg	300 min
Rat Anti-Mouse IL-5	RM9060	Pur. Ab.	1.0 ml	200 µg	
CloneTRFK5	RM9061	FITC	1.0 ml	100 µg	100 min
Rat IgG1	RM9061-3	FITC	3.0 ml	300 µg	300 min
	RM9064	R-PE	0.5 ml	50 µg	50 min
	RM9064-3	R-PE	3.0 ml	300 µg	300 min
Rat Anti-Mouse IL-10	RM9100	Pur. Ab.	1.0 ml	200 µg	
Clone JES5-2A5	RM9101	FITC	1.0 ml	100 µg	100 min
Rat IgG1	RM9101-3	FITC	3.0 ml	300 µg	300 min
	RM9104	R-PE	0.5 ml	50 µg	50 min
	RM9104-3	R-PE	3.0 ml	300 µg	300 min
Rat Anti-Mouse TNF-α	RM9010	Pur. Ab.	1.0 ml	200 µg	
Clone MP6-XT22	RM9011	FITC	1.0 ml	100 µg	100 min
Rat IgG1	RM9011-3	FITC	3.0 ml	300 µg	300 min
	RM9014	R-PE	0.5 ml	50 µg	50 min
	RM9014-3	R-PE	3.0 ml	300 µg	300 min

Reagents Available From 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 µg	
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 µg	
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 µg	
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 µg	
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 µg	
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 µg	
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 µg	
Clone VL1	RANT101	FITC	1.0 ml		100 min
Rat IgG2b	RANT104	R-PE	1.0 ml		100 min

Reagents Available From CALTAG

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	MHBCL01	FITC	0.5 ml	100 min
Clone 100	MHBCL01-4	FITC	2.0 ml	400 min
Mouse IgG1	MHBCL04	R-PE	0.5 ml	100 min
Mouse Anti-Human PCNA	PCNA01	FITC	0.5 ml	100 min
Clone PC10	PCNA04	R-PE	0.5 ml	100 min
Mouse IgG2a				
Mouse Anti-Human κ	MH10520	Alexa 488	0.5 ml	100 min
Clone HP6062	MH10511	FITC	0.5 ml	100 min
Mouse IgG3	MH10514	R-PE	0.5 ml	100 min
Mouse Anti-Human λ	MH10620	Alexa 488	0.5 ml	100 min
Clone HP6054	MH10611	FITC	0.5 ml	100 min
Mouse IgG2a	MH10614	R-PE	0.5 ml	100 min
Goat F(ab')2 Anti-Human κ	H16101	FITC	1.0 ml	700 min
	H16104	R-PE	0.5 ml	100 min
Goat F(ab')2 Anti-Human λ	H16601	FITC	1.0 ml	700 min
	H16604	R-PE	0.5 ml	100 min
Goat F(ab')2 Anti-Human IgG	H10101	FITC	1.0 ml	700 min
(γ chain)	H10104	R-PE	0.5 ml	100 min
Goat F(ab')2 Anti-Human IgM	H15101	FITC	1.0 ml	700 min
(μ chain)	H15104	R-PE	0.5 ml	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

Selected References

SELECTED REFERENCES FOR INTRACELLULAR ANTIGEN DETECTION

1. Knapp, W., Madjic, O. and Strobl H.

   Flow Cytometric Analysis of Intracellular Myeloperoxidase and Lactoferrin in Lekemia Diagnosis.
   Recent Results Cancer Res. 131: 31-40 (1993).

2. Strobl, H, Takimoto, M., Madjic, O., Fritsch, G., Scheinecker, C., Höcker and Knapp, W.

   Myeloperoxidase Expression in CD34+ Normal Human Hemopoietic Cells.
   Blood 82: 2069-2078 (1993).

3. Knapp, W., Strobl, H. & Madjic, O.

   Flow Cytometric Analysis of Cell Surface and Intracellular Antigens in Leukemia Diagnosis.
   Cytometry (Communications in Clinical Cytometry) 18: 187-198 (1994).

4. Ferrick, D.A., Schrenzel, M.D., Mulvania, Th., Hsieh, B., Ferlin, W.G. and Lepper, H.

   Differential Production of Interferon-γ and Interleukin-4 in Response to Th1 and Th2-stimulating pathogens by $\gamma\delta$ T cells in vivo.
   Nature 373: 255-257 (1995).

5. Knapp, W., H. Strobl, C. Scheinecker, C. Bello-Fernandez and O. Madjic

   Molecular Characterization of CD34+ Human Progenitor Cells.
   Ann. Haematol. 70: 281-296 (1995).

6. Koch, F., B. Trockenbacher, I. Kämpgen, O. Grauer, H. Stössel, A.M. Livingstone, G. Schuler and N. Romani

   Antigen Processing in Populations of Mature Murine Dendritic Cells Is Caused By Subsets of Incompletely Matured Cells.
   J. Immunol 155: 93-100 (1995).

7. Scheinecker, C., H. Strobl, G. Fritsch, B. Csmarits, O. Krieger, O.,Madjic and W. Knapp

   Granulomonocyte-Associated Lysosomal protein expression during in vitro expansion and differentiation of CD34+ hemopoietic Progenitor Cells.
   Blood 86: 4115-4123 (1995).

8. Strobl, H., C. Scheinecker, B. Csmartis, O. Madjic and W. Knapp

   Flow Cytometric Analysis of Intracellular CD68 molecule expression in normal and malignant haematopoiesis.
   Brit. J. Haematol. 90: 774-782 (1995).

9. M.E. North, K. Ivory et al:

   Intracellular cytokine production by human CD4+ and CD8+ T cells from normal and immunodeficient donors using directly conjugated anti-cytokine antibodies and flow cytometry..
   Clin. Exp. Immunol. 105: 517-522 (1996).

10. Sedlmayer, P., B. GroBhaupt and W. Muntean

    Flow Cytometric Detection Of Intracellular Platelet Antigens.
    Cytometry 23: 284-289 (1996).

11. Murao, S.I., Stevens, F.J., Ito, A. and Huberman, E.

    Myeloperoxidase: a Myeloid Cell Nuclear Antigen with DNA-Binding Properties.
    Proc. Nat Acad Sci. USA 85: 1232-1236 (1988).

12. Koeffler, H.P., Ranyard, J., and Pertcheck, M.

    Myeloperoxidase: Its Structure During Myeloid Differentiation.
    Blood 65: 484-491 (1988).

13. Campana, D., Thompson, J.S., Amlot, P., Brown, S. and Janossy, G.

    The Cytoplasmic Expression of CD3 Antigens in Normal and Malignant Cells of the T Lymphoid Lineage.
    J. Immunol. 138: 684-655 (1987).

14. Janossy, G, Coustan-Smith, E. and Campana, D.

    The Reliability of Cytoplasmic CD3 and CD22 Antigen Expression in the Immunogiagnosis of Acute Leukemia: A Study of 500 Cases.
    Leukemia 3: 170-181 (1989).

15. Groeneveld, K., JG te Marvelde, MWM van den Beemd, H. Hooijkaas and JJM van Dongen.

    Flow cytometric determination of intracellular antigens for immunophenotyping.
    Leukemia 10: 1383-1389 (1996).

16. B. Meenan, C. Heavey, A. Lichtenstein, J. Andersen & E. Paietta:

    Terminal Transferase Expression in the Differential Diagnosis of Acute Leukemias.
    Leukemia and Lymphoma 22: 265-269 (1996).

SELECTED REFERENCES FOR INTRACELLULAR ANTIGEN DETECTION (CONTINUED)

17. Francesco Lanza et al:

    Comparative Analysis of Different Permeabilization Methods for the Flow Cytometry Measurement of Cytoplasmic Myeloperoxidase and Lysozyme in Normal and Leukemic Cells.
    Cytometry (Communications in Clinical Cytometry) 30: 134-144 (1997).

18. E. Colombo:

    CMV Ag Assay by Flow Cytometry.
    Personal Communication to Purdue Flow Cytometry Bulletin Board, Hospital-Como (coledoco@tin.it)

19. Strobl, H., E. Riedl, C. Scheinecker, C. Bello-Fernandez, W.F. Pickl, K. Rappersbeger, O. Madjic and W. Knapp

    TGF- 1 promotes in vitro development of dendritic cells from CD34+ hemopoietic progenitors
    J. Immunol. 157: 1499-1507 (1996).

20. Strobl, H., C. Bello-Fernandez, E. Riedl, W.F. Pickl, O. Madjic, S.D. Lyman and W. Knapp

    flt3 ligand in cooperation with TGF- 1 potentiates in vitro development of Langerhans type dendritic cells and allows single cell DC cluster formation under serum-free conditions.
    Blood 90: 1425-1434 (1997).

21. Garcia Vela, J.A. et al

    Acute myeloid leukemia M2 and t (8;21) (q22;q22) with an unusual phenotype: myeloperoxidase (+) CD13 (-), CD14 (-) and CD33 (-)
    Ann. Hematol. 78: 237-240 (1999).

22. Urbano-Ispizua, A. et al

    The value of detecting surface and cytoplasmic antigens in acute myeloid leukaemia
    Brit. J. Haematol. 81: 178-183 (1992).

23. Kaefer, G. et al

    Intracellular expression of CD61 precedes surface expression
    Ann. Haematol. 78: 472-474 (1999).

24. Farrant, J.et al

    Measurements of intracellular cytokines in normal and immunodeficient lymphocytes using directly conjugated antibodies.
    Cytometry Suppl. 8,98 (Abstract) (1996).

25. Fleisher, T.A. et al Detection of Intracellular Phosphorylated STAT-1 by Flow Cytometry.
    Clinical Immunology 90: 425-430 (1999).

26. Porwit-MacDonald, A. et al

    Flow cytometric characterization of CD7+ cell subsets in a normal bone marrow as a basis for the diagnosis and follow-up of T cell acute lymphoblastic leukemia.
    Leukemia 14: 816-825 (2000).

27. San Miguel, J.F. et al

    Immunophenotyping investigation of minimal residual disease is a useful approach for predicting relapse in acute myeloid leukemia patients.
    Blood 90: 2465-2470 (1997).

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