HotLines

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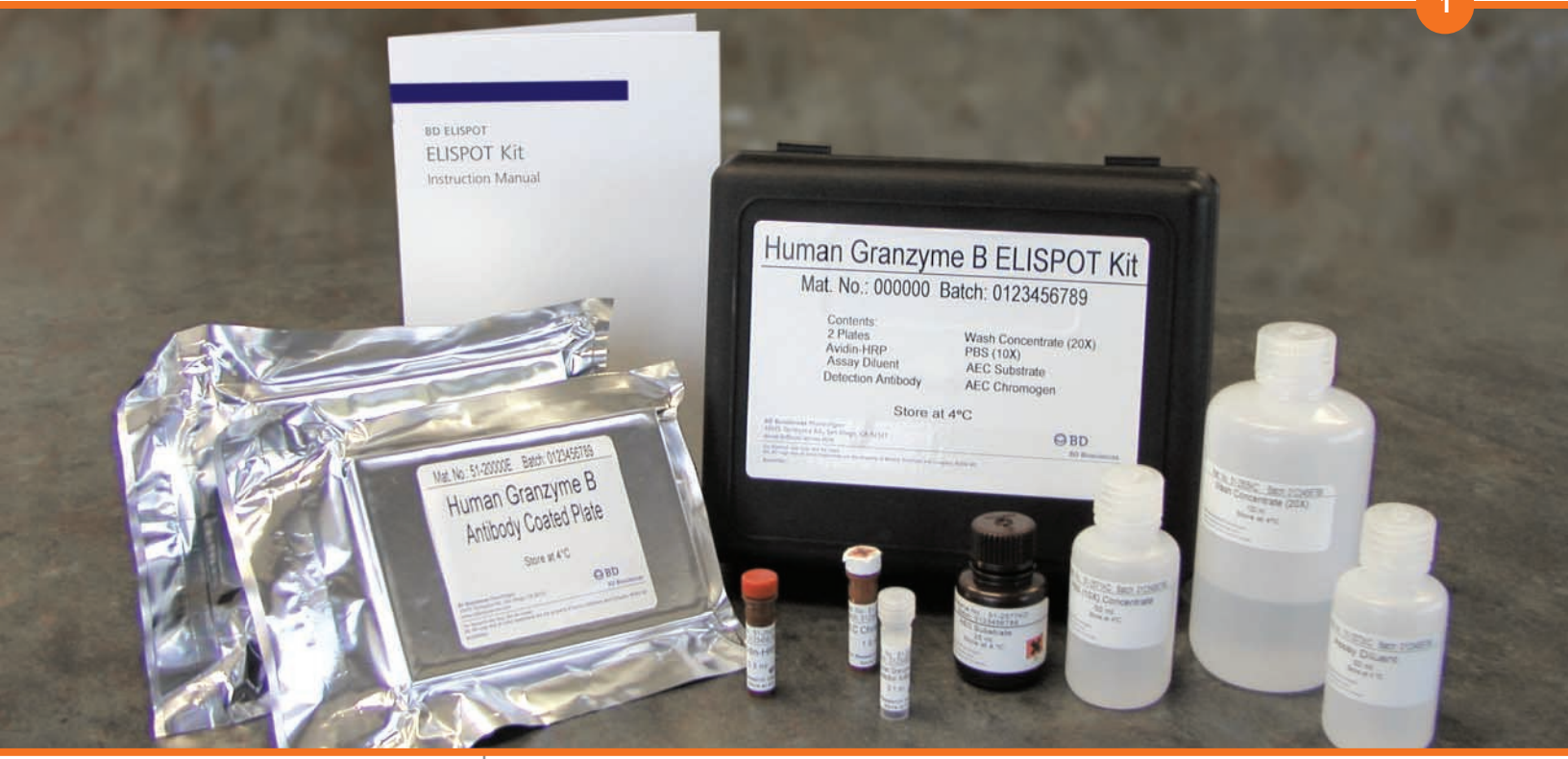

IN THIS ISSUE:

Analysis of Cell-mediated Immunity by Granzyme B ELISPOT Technology

By Qi Guan, Wingman Ma, David Ernst, PhD, Edward Morgan, PhD, and Jay Dong, MD, MS

Building upon our extensive portfolio of techniques, tools, and solutions for immune function analysis and apoptosis research, BD Biosciences Pharmingen is pleased to introduce the BD™ ELISPOT Human Granzyme B assay.

Continued on page 2

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BD

Granzyme B ELISPOT Technology (continued from cover)

A non-radioactive alternative to Chromium-release assays with superior signal-to-noise ratios for the detection of cytolytic cells

Cytotoxic granules secreted by cytolytic T lymphocytes and natural killer (NK) cells are part of the mechanism that protects organisms from virus-infected cells and tumor cells. Granzyme B, a prominent granzyme, is secreted by cytolytic effector cells that target cells through transmembrane pores formed by another granule protein, perforin.

In the target cell, granzyme B acts on specific substrates involved with programmed cell death via apoptosis.[1] Granzyme B is a neutral serine protease that induces apoptosis by cleaving and activating members of the caspase family. The detection of Granzyme B-secreting cells in ELISPOT assays correlates with cytolytic responses measured by the classic radioactive chromium (^{51}Cr)-release assay.[4] Compared with cytolytic cell responses measured by ^{51}Cr-release assays, BD ELISPOT Granzyme B assays typically yield superior signal-to-noise ratios when analyzing responses by human peripheral blood mononuclear cells (HPBMCs). Furthermore, the BD ELISPOT Granzyme B assays avoid the use of potentially hazardous and difficult-to-dispose-of radioactive labels used in ^{51}Cr-release assays.[2]

Figure 1. BD ELISPOT Human Granzyme B Kit. *The kit components include two pre-coated plates and all necessary reagents: Antibody-coated Plates, Detection Antibody, Avidin-HRP, AEC Substrate/Chromogen, Assay Diluent, PBS Concentrate and Wash Concentrate.*

The BD ELISPOT Granzyme B assay is highly sensitive and suitable for large-scale testing of T cell reactivities using peptides.

The BD ELISPOT Granzyme B assay shows high sensitivity in detecting molecules secreted by individual cells that are present in low frequencies. For this reason, the assay is suitable for large-scale testing of peptide reactivities demonstrated by freshly isolated HPBMC populations.[2]

The BD ELISPOT Granzyme B assay directly measures the frequencies of Granzyme B-producing cells. With high-throughput ELISPOT plate-reader instrumentation, objective and rapid analyses of Granzyme B-producing cell numbers (spots) and relative amounts of Granzyme B produced per cell (spot size) are now a reality.

BD ELISPOT Granzyme B Kits contain pre-coated plates manufactured by an automated coating process that ensures consistent results

Just like our BD ELISPOT Human IFN-γ Kits, the Immunospot™ plates included in BD ELISPOT Human Granzyme B Kits are coated by BD Biosciences Discovery Labware, the leader in biological coating, to ensure consistent results with minimal variation. BD Biosciences Pharmingen is pleased to introduce the BD ELISPOT Human Granzyme B Kit with pre-coated plates (please inquire) and Set (Cat. No. 552572) for measuring the frequencies of Granzyme B-secreting cells directly.

The BD ELISPOT Human Granzyme B Set was used to analyze the nature of normal and primed HPBMCs that secrete Granzyme B in response to PMA and ionomycin stimulation. The results show that when freshly prepared, resting HPBMCs are stimulated with PMA/ionomycin, a number of HPBMCs are stimulated to produce Granzyme B (Figure 2A) at different levels (Figure 2B). When the HPBMCs are first primed *in vitro* in a system designed to model *in vivo* activation and differentiation, the frequency of Granzyme B-secreting cells is significantly increased (Figure 2C) with a shift to a higher frequency of cells that secrete higher levels of Granzyme B (Figure 2D).

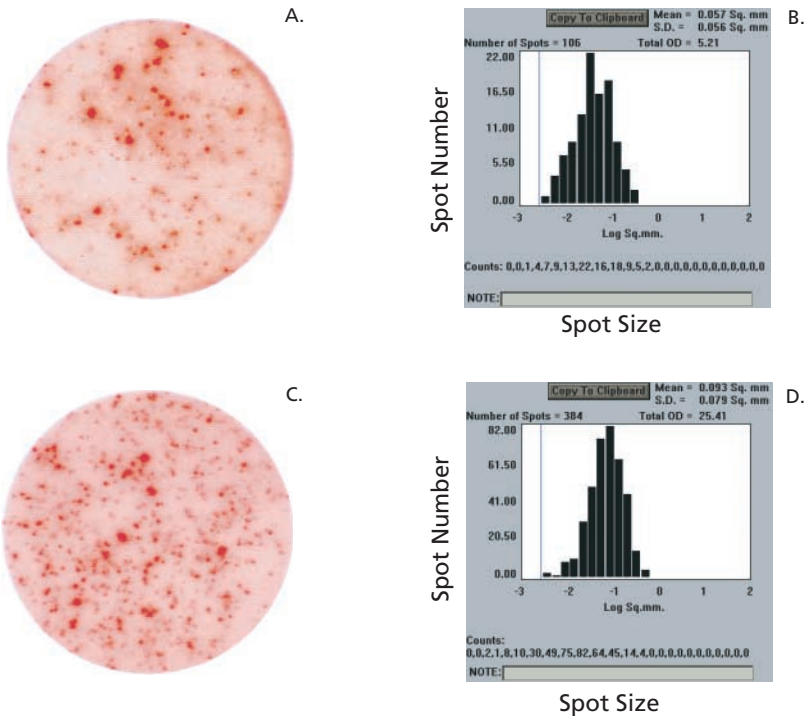

Figure 2. Analysis of Granzyme B-secreting cells arising from stimulated resting or in vitro primed Human PBMC using the BD ELISPOT Granzyme B Set. *Freshly-prepared HPBMCs were stimulated with PMA/ionomycin (18 hr) in an ImmunoSpot ELISPOT plate whose well membranes were pre-coated with a purified NA/LE anti-human Granzyme B capture antibody. After the cells were washed away, the resulting ELISPOTS were revealed by using a biotinylated anti-human Granzyme B detection antibody, avidin-HRP and AEC substrate/chromogen followed by analysis of the ELISPOT image (Panel A) and spot enumeration and sizing using an ImmunoSpot® Series I Analyzer. 106 Granzyme B-secreting cells/5 × 10^4 plated cells were detected. The frequency distribution for the spot sizes generated by PMA/ionomycin-activated cells, as measured by image analysis, is shown in Panel B.*

In a separate experiment, resting HPBMCs were stimulated with immobilized anti-human CD3 and soluble anti-human CD28 in the presence of recombinant human IL-2 and IL-4 for 2 days. The cells were washed and subsequently cultured in medium containing recombinant human IL-2 and IL-4 for 3 days. The cells were harvested, plated at different densities, and were then stimulated with PMA/ionomycin (15 hr) and tested for Granzyme B-secretion. The analysis of the image (Panel C) revealed a significantly increased frequency of Granzyme B-secreting cells (384 Granzyme B-secreting cells/104 plated cells) that secrete higher levels of Granzyme B (Panel D) from an in vitro-primed HPBMC population.

References:

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BD ELISPOT Human Granzyme B reagents

DESCRIPTION	SIZE	CAT. NO.
BD ELISPOT Human Granzyme B Kit	2 pre-coated plates and all reagents needed	**inquire**
BD ELISPOT Human Granzyme B Set	10 uncoated plates, capture Ab, detection Ab, AV-HRP	**552572**

For a complete listing of all BD ELISPOT products as well as other products for immune function analysis, please visit www.bdbiosciences.com/immune_function/

ImmunoSpot Series 2.0 Analyzer

Now Available – Series 2.0 ImmunoSpot Analyzer and ImmunoSpot version 2.0 Software for Automated, High Throughput ELISPOT Data Acquisition and Analysis

BD Biosciences and our ELISPOT instrumentation and software partner Cellular Technology Limited (Cleveland, OH) are proud to announce the launch of the next generation ELISPOT image analysis system and software, which are scientifically validated for single-cell resolution. The ImmunoSpot Series 2.0 Analyzer can come equipped with software features such as: ImmunoSpot® Multi-Plate AutoCount™ (IMPAC™), which permits uninterrupted, walk-away counting of an open number of plate image files; ImmunoSpot Map™, a data management tool for high volume data processing, and ImmunoSpot QC™, a quality control tool for data validation. The ImmunoSpot Series 2.0 Analyzer is ideal in a core facility setting as a shared resource; a single Analyzer can support a large number of satellite workstations, enabling independent data analysis at multiple sites.

A new approach to establish full ELISPOT image analysis capabilities at a small fraction of the price of any available ELISPOT reader is the Satellite & Scanning Service Package. This package is designed for laboratories whose workload or budget does not yet justify purchasing an Analyzer, but who wish to establish high capacity, fully independent ELISPOT analysis capabilities immediately. This package includes a high end ImmunoSpot Satellite workstation combined with unlimited free scanning of your ELISPOT plate data to CD or DVD by our laboratories. For further information on the Satellite & Scanning Service Package or the Free ImmunoSpot Software Trial, please visit **www.immunospot.com** or contact your local BD Biosciences Reagent Sales Representative.

C.T.L.
Cellular Technology Ltd.

ImmunoSpot ELISPOT plates and ImmunoSpot Series I Analyzer are trademarks of Cellular Technology Limited, Cleveland, Ohio.

The BD™ ELISPOT Reagent Sets and protocols were developed in collaboration with Cellular Technology Limited, Cleveland, Ohio.

BD IMag™ Cell Separation System

By Jennifer Bickel, Alejandro Uribe, Peter Li, and Jurg Rohrer

Magnetic Cell Separation

Magnetic cell separation technology allows for the rapid selection and separation of cell subpopulations. The separation protocol can be divided into three basic steps:

1. The target cells are labeled with antibody-conjugated magnetic particles.
2. The labeled cells are placed within a magnetic field.
3. The labeled cells are retained within the field while the unlabeled cells are washed out (Figure 1).

Several technologies are currently available, with the primary difference between the technologies being particle size. BD Biosciences Pharmingen has developed the BD™ IMag magnetic cell separation system, which uses approximately 200-nm size particles. Because of the particle size, the BD IMag system can take advantage of simple direct magnet technology to separate labeled cells in test tubes instead of using magnetic separation columns. In addition, the particles are small enough to be compatible with flow cytometry and make it possible to culture positively selected cells post-separation.

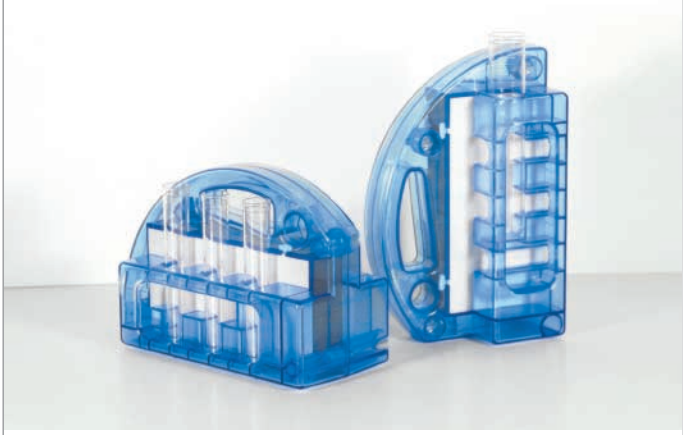


Figure 2. The BD™ IMagnet is a neodymium iron boron permanent magnet that can hold either six BD Falcon™ 12 × 75 mm test tubes (Cat. No. 352008) or two BD Falcon 17 × 100 mm test tubes (Cat. No. 352057).

To offer users additional flexibility for cell separations, two types of BD IMag products are available. These include products for use with the BD IMagnet, a direct magnet system (DM products), and products for use with existing magnetic separation column systems (MSC products). Combined with the diversity and high quality of BD Pharmingen™ monoclonal antibodies, the BD IMag cell separation system offers researchers an affordable and easy-to-use tool for a wide range of cell separation applications.

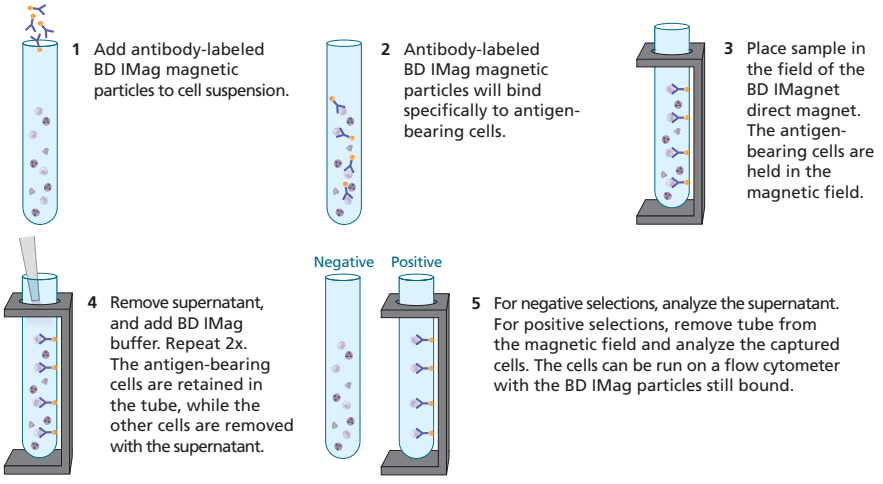


Figure 1. The BD IMag cell separation procedure using the BD IMagnet direct magnet and BD IMag Particles-DM.

BD IMag™ Cell Separation System (continued from page 5)

Isolation of mouse CD11b⁺ cells and subsequent culture of dendritic cells

The BD IMag anti-mouse CD11b particles - DM can be used for the positive selection or depletion of mouse CD11b⁺ cells. CD11b⁺ cells were selected from BALB/c bone marrow and the purity of the negative and positive fractions were assessed by flow cytometry (Figure 3). To culture dendritic cells, positively selected cells were placed into culture with GM-CSF for 6 days followed by a 24-hour stimulation with LPS. As expected, at the end of the culture period, cells expressed CD11b, and the dendritic cell marker CD11c (Figue 4). Expression of CD8 was also seen on a sub-population of CD11c⁺ cells (Figure 4). In addition, expression of DEC205 was confirmed as was the upregulation of B7-1 (CD80) and MHC class II (data not shown).

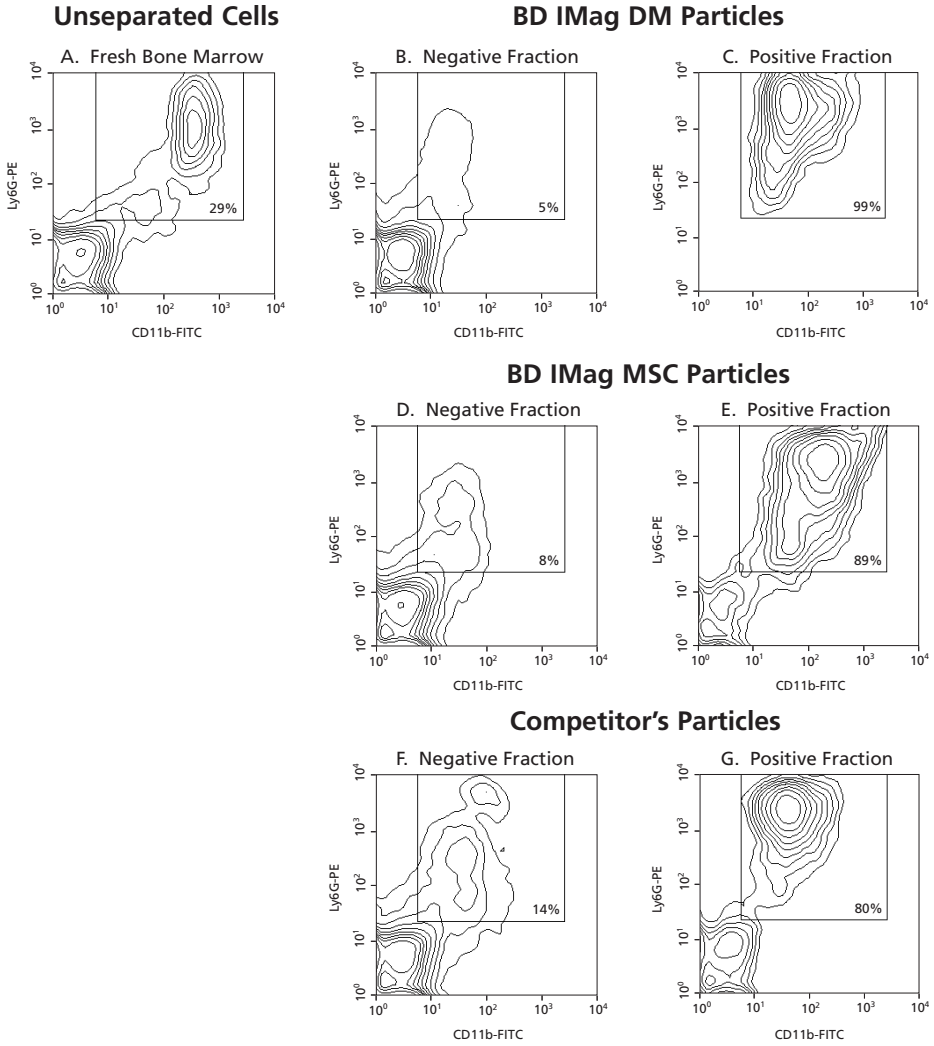


Figure 3. Bone marrow cells were labeled with BD IMag anti-mouse CD11b particles - DM and MSC. After labeling, the cells were separated into negative (CD11b⁻) and positive (CD11b⁺) fractions, using either the BD IMagnet or a magnetic separation column. A separation using a competitor's CD11b magnetic particles and the magnetic separation column procedure is also shown. For flow cytometric analysis, unmanipulated cells (A), the negative fractions from the direct magnet (B), the magnetic separation column (D), and the competitor (F), and the corresponding positive fractions were stained with anti-CD11b-FITC (clone M1/70) and anti-Ly6G-PE (clone RB6-8C5). The percent CD11b positive cells in each sample is shown.

Isolation and tissue culture of human CD3+ T cells

The BD IMag anti-human CD3 particles - DM can be used for the positive selection or depletion of human CD3+ T cells. For this experiment, cells were positively selected from Ficoll Hypaque purified peripheral blood mononuclear cells (PBMCs). The yield of the positive and negative fractions was determined and purity of the two fractions was assessed by flow cytometry (Figure 5). Positively selected cells were placed into culture and either stimulated with plate bound anti-human CD3 and CD28 or plate bound anti-human CD3 alone. Upregulation of CD25 and CD69 were monitored 24 hours later and proliferation was determined by H^3 thymidine incorporation 48 hours later (Figure 6).

In summary, the BD IMag anti-human CD3 particles - DM can be used to effectively deplete or enrich CD3+ cells from a sample. Positively selected cells can subsequently be placed into culture. Cultured cells respond to stimuli and upregulate activation markers and incorporate H^3-thymidine as expected. Of note, the particles have been optimized to deplete CD3+ cells from a donor that has 60% to 70% CD3+ T cells. Should the donor have fewer CD3+ T cells, then fewer particles can be used.

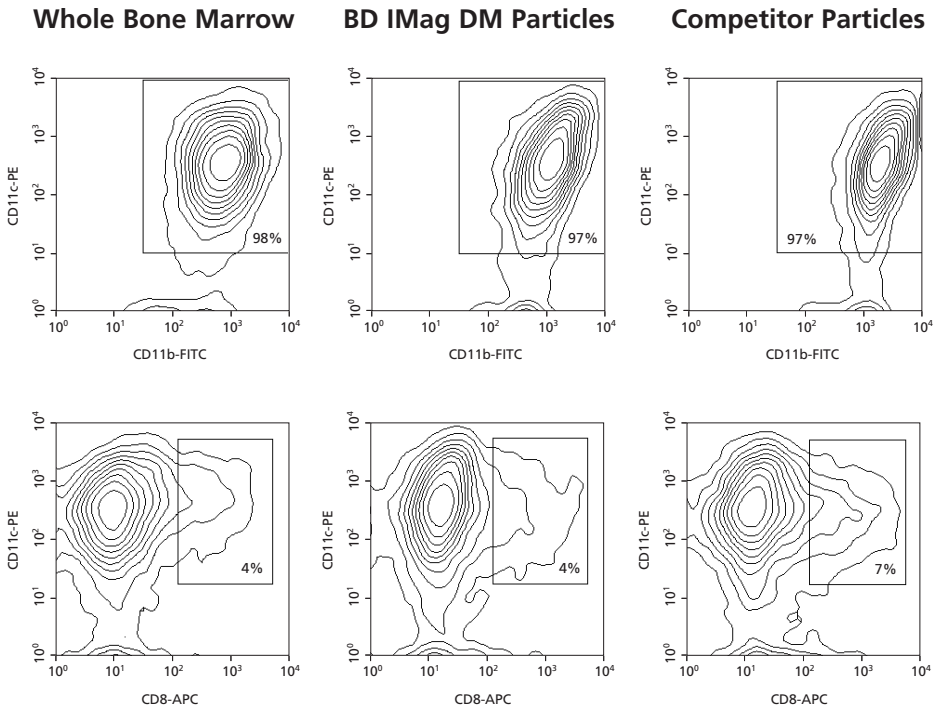


Figure 4. Dendritic cells were generated from whole mouse bone marrow and the positively selected (CD11b+) cells. Whole bone marrow and CD11b+ cells were cultured for 6 days with 1000 U of mouse GM-CSF followed by a twenty-four hour stimulation with LPS. For flow cytometric analysis, cells from the whole bone marrow culture (A), cells cultured after positive selection with the BD IMag anti-mouse CD11b particles - DM (B), and cells cultured after positive selection with a competitor's particles (C) were stained with anti-CD11b FITC (clone M1/70) and anti-CD11c PE (clone HL3). The percent CD11b+ CD11c+ cells in each sample is shown. A subset of CD11c+ cells are CD8+, on cultured cells from whole bone marrow (D), and cells isolated using the CD11b - DM particles (D), and a competitor's particles (F). The percent CD11c+ CD8+ cells in each sample is shown.

BD IMag™ Cell Separation System *(continued from page 7)*

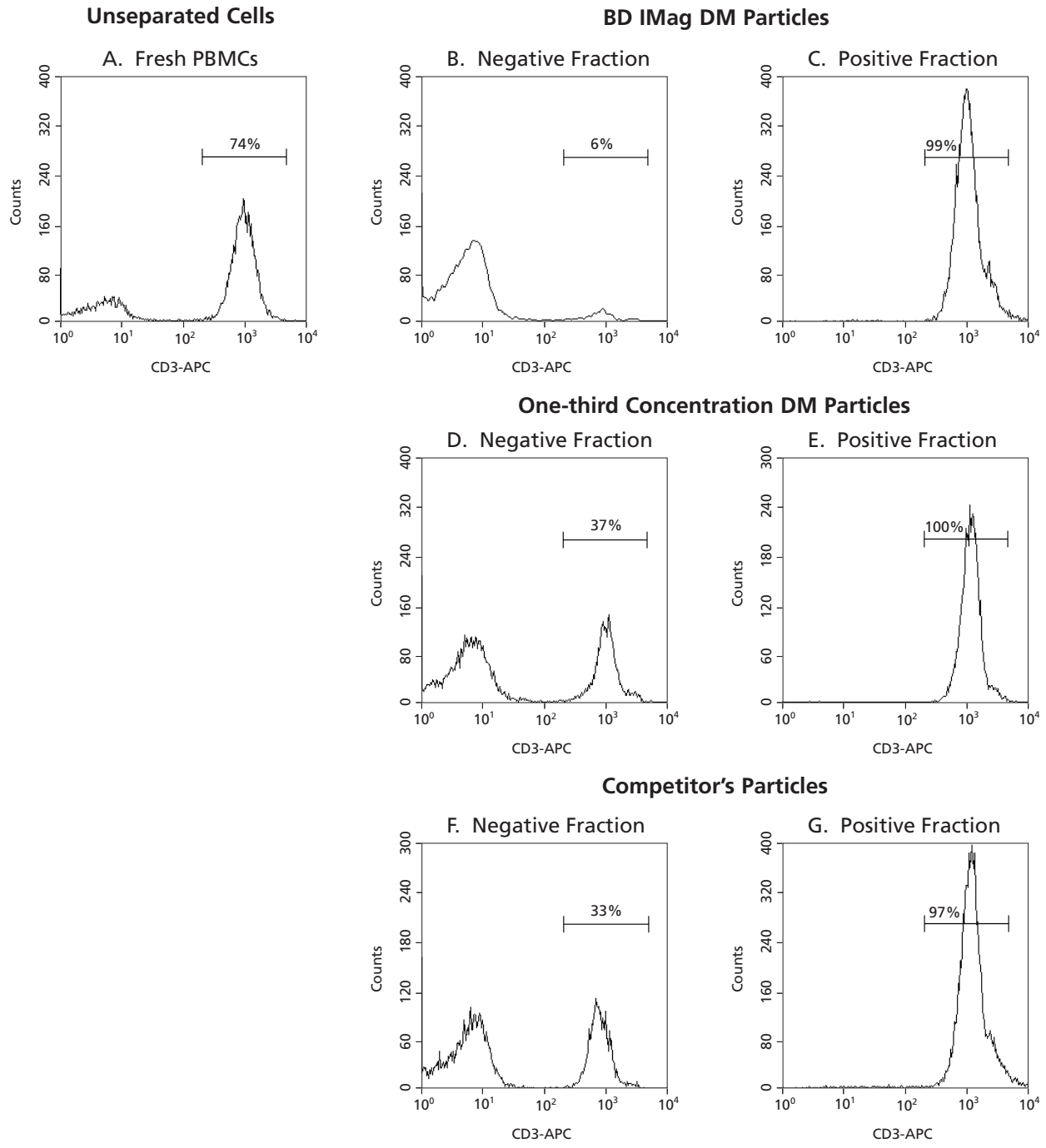
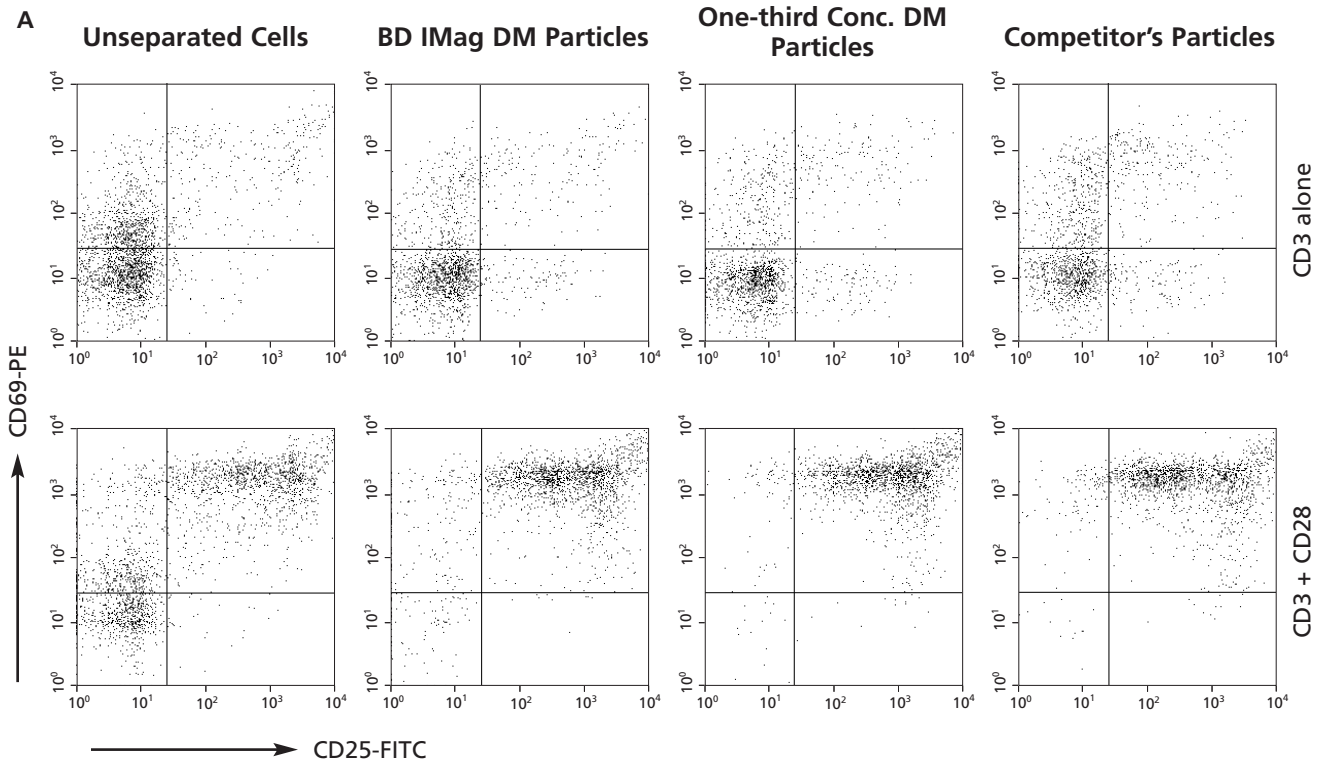


Figure 5. *Leukocytes were labeled with BD IMag anti-human CD3 particles - DM using the particles at both the recommended concentration or at one-third the recommended concentration. After labeling, the cells were separated into negative ($CD3^-$) and positive ($CD3^+$) fractions, using the BD IMagnet. A separation using a competitor's magnetic particles and the magnetic separation column procedure is also shown. For flow cytometric analysis, unmanipulated cells (A), the negative fractions from the BD IMagnet separations (B) and (D) and the competitor (F), and the positive fractions from the BD IMagnet separations (C) and (E) and the competitor (G) were stained with anti-CD3-APC (clone UCHT1). The percent $CD3^+$ cells in each sample is shown.*



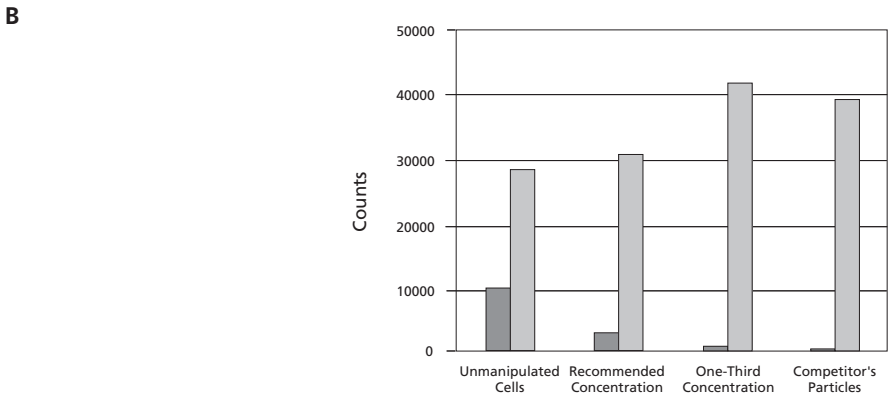


Figure 6. *CD3⁺ cells were plated in 96 well plates that had been pre-coated with either anti human CD3 alone (clone UCHT1) at 10ng/ml or anti-human CD3 and anti-human CD28 (clone CD28.2) at 2µg/ml. Cells were cultured for 48 hours and then analyzed by flow cytometry for the expression of CD25 and CD69 (A) or pulsed with H³-thymidine for 8 hours, at which time H³-thymidine incorporation was measured (B).*

Figure 6A. *Upregulation of CD25 (clone M-A251) and CD69 (clone FN50) on unmanipulated cells compared to CD3⁺ cells isolated using the BD IMag anti-human CD3 particles - DM and a competitor's particles. While upregulation of CD25 and CD69 is minimal in the CD3 only cultures, both markers are comparably upregulated for all three conditions, the two DM separations done with particles used at the recommended concentration and at one-third the recommended concentration and the competitor's particles.*

Figure 6B. *H³-thymidine incorporation is shown for the CD3 alone and the CD3/CD28 cultures. In all cases there was a significant difference between the CD3 only cultures (darkly shaded bars) and the CD3/CD28 co-cultures (lightly shaded bars). For some donors (as is shown here) there is a difference between cells that were isolated using the BD IMag anti-human CD3 particles - DM at the recommended versus one-third the recommended concentration.*

BD IMag™ Products Available *(continued from page 9)*

DESCRIPTION	CLONE	SIZE	CAT. NO.
BD IMag Streptavidin Particles - DM*		$2x10^9$ cells	**551307**
BD IMag Streptavidin Particles - MSC**		$2x10^9$ cells	**551308**
BD IMagnet (holds six 12x75 mm test tubes or two 17x100 mm test tubes)			**551768**
Mouse			
BD IMag anti-mouse CD4 Particles - DM	GK1.5	$2x10^9$ cells	**551539**
BD IMag anti-mouse CD4 Particles - MSC	GK1.5	$2x10^9$ cells	**551540**
BD IMag anti-mouse CD8a Particles - DM	53-6.7	$2x10^9$ cells	**551516**
BD IMag anti-mouse CD8a Particles - MSC	53-6.7	$2x10^9$ cells	**551517**
BD IMag anti-mouse CD45R/B220 Particles - DM	RA3-6B2	$2x10^9$ cells	**551513**
BD IMag anti-mouse CD45R/B220 Particles - MSC	RA3-6B2	$2x10^9$ cells	**551514**
BD IMag anti-mouseCD90.2 (Thy1.2) Particles - DM	30-H12	$2x10^9$ cells	**551518**
BD IMag anti-mouse CD90.2 (Thy1.2) Particles - MSC	30-H12	$2x10^9$ cells	**551519**
Human			
BD IMag anti-human CD19 Particles - DM	HIB19	$1x10^9$ cells	**551520**
BD IMag anti-human CD19 Particles - MSC	HIB19	$1x10^9$ cells	**551521**
Products Coming Soon:			
BD IMag anti-human CD3 particles - DM and MSC			
BD IMag anti-human CD4 particles - DM and MSC			
BD IMag anti-human CD8 particles - DM and MSC			
BD IMag anti-human CD14 particles - DM and MSC			
BD IMag anti-mouse CD11b particles - DM and MSC			
BD IMag anti-mouse Ly6G particles - DM and MSC			
BD IMag mouse lineage progenitor enrichment kit - DM and MSC			

**DM - Optimized for use with the BD IMagnet Direct Magnet*
***MSC - Optimized for use with Magnetic Separation Columns*

BD IMag particles are prepared from carboxy-functionalized magnetic particles which are manufactured by Skold Technology

Custom Peptide Mixes for Cytokine Flow Cytometry

By Kerstin Willmann, Jerome A. Zawadzki, and Holden T. Maecker

Introduction

Mixtures of overlapping peptides have been increasingly used for *in vitro* stimulation of T cells.[1-3] Stimulated T cells can be measured and enumerated by cytokine flow cytometry (CFC), ELISPOT, or other monitoring assays. CFC is a particularly beneficial technology as it allows one to differentiate responses of CD4 and CD8 T cells, which are readily resolved by the multiparametric nature of flow cytometry.

Overlapping peptide mixtures have several benefits over the use of whole protein and whole viral lysate preparations. First, because they are synthetically derived, they can be made reproducibly, with less potential lot-to-lot variability. Second, whole protein antigens preferentially stimulate MHC class II–restricted $CD4^+$ T-cell responses.[4,5] This is because of the tendency of exogenous antigens to be processed for presentation by MHC class II–molecules. By carefully choosing the peptide length, overlapping peptide mixtures can stimulate both CD4 and CD8 responses in the same sample.

Mixtures of 15-mer peptides that overlap by 11 amino acid residues each can span an entire immunogenic protein so that all possible epitopes of 9 amino acid residues are contained in at least one peptide of the mixture. The mixture can then be used as a single antigen for short *in vitro* restimulation (about 6 hours) of peripheral blood mononuclear cells (PBMCs) or whole blood. Alternately, smaller pools of overlapping peptides can be created using a *matrix* approach, so that epitopes can be rapidly mapped.[1,6]

The synthetic peptide mixtures bind to extracellular MHC molecules and directly stimulate T cells. CD8 T cells respond to peptides that are 8–10 amino acid residues in length. The binding groove of MHC class I–molecules has *closed* ends that make it difficult for longer peptides to bind.[7,8] CD4 cells can respond to peptides of 10 amino acid residues or longer, as the binding groove of MHC class II–molecules has open ends that allow longer peptides to protrude.[9] Despite these differences in length requirements for MHC class I– and MHC class II–binding, peptides of 15 amino acid residues have been shown to efficiently stimulate both CD4 and CD8 responses.[3] The mechanism by which 15-mer peptides can elicit CD8 responses is not fully understood, although the responses can be shown to be MHC class I–restricted. It is assumed that extracellular processing by serum or cell-associated proteases, or both, plays a role. However, this mechanism appears to be limited, as 20-mer peptides are less efficient than 15-mers at inducing CD8 responses[3] (see Figure 1).

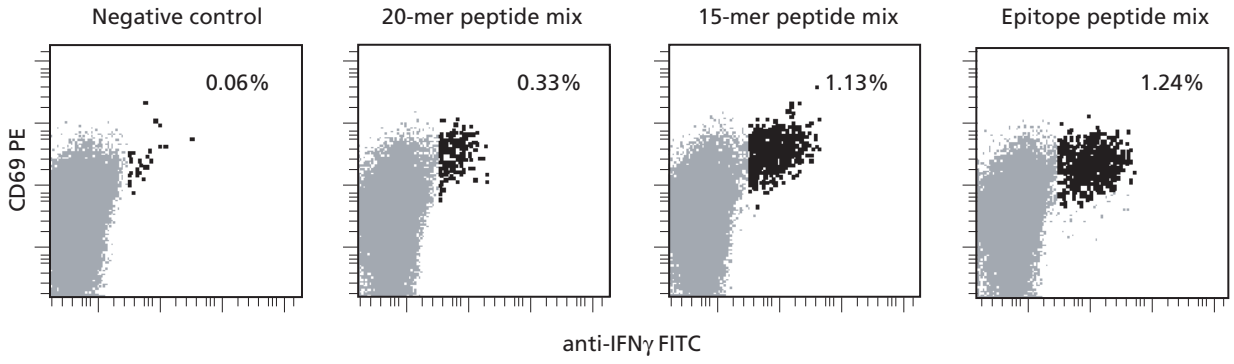


Figure 1. *Comparison of CD8 responses using an overlapping peptide mix of 20-mer peptides, 15-mer peptides, or known 8–12 amino acid epitopes from HIV p55 gag, in an HIV seropositive donor.*

Custom Peptide Mixes for Cytokine Flow Cytometry (continued from page 11)

Materials and Methodology

BD FastImmune™ CD8 (IFN-γ, four-color) and CD4 (IFN-γ, IL-2, or TNF-α, three-color) Cytokine Detection Kits readily identify CD4 and CD8 responses in human whole blood or PBMC samples stimulated with peptide mixes.* The kits make getting started easier with a simplified protocol and optimized staining and sample processing reagents (see Figure 2). The three-color BD FastImmune CD4 Kit Multicolor Cocktail (Anti-Cytokine FITC/CD69 PE†/CD4 PerCP-Cy5.5†) can be supplemented with CD3 APC† to allow further discrimination of $CD3^+CD4^+$ cells from $CD3^+CD4^-$ cells. The latter population is roughly equivalent to direct detection of $CD3^+CD8^+$ cells. However, $CD4^+CD8^{dim}$ T cells (which are MHC class II–restricted[10]) will be included in the $CD3^+CD8^+$ population, but not the $CD3^+CD4^-$ population. Please note that the BD FastImmune system is highly optimized to allow for rare event detection and should not be mixed with other reagent products.

Kits contain:

- BD FastImmune Anti-Hu–IFN-γ FITC/CD69 PE†/CD8 PerCP-Cy5.5†/CD3 APC†
  Or BD FastImmune Anti-Hu–cytokine FITC/CD69 PE/CD4 PerCP-Cy5.5
- BD FastImmune matching multicolor isotype control
- BD FastImmune Brefeldin A
- BD FastImmune EDTA Solution
- BD FastImmune CD28/CD49d Costimulatory Reagent
- BD FACS™ Lysing Solution†
- BD FACS™ Permeabilizing Solution 2

Note: All kit components are also available individually.

In addition to the ability to simultaneously detect CD4 and CD8 responses in a single sample, peptide mixes also allow for better detection of responses in cryopreserved PBMCs or shipped whole blood.[3] This seems to reflect the fact that antigen-presenting cells, such as monocytes, are more affected by shipping, or freezing or thawing, or both, than are T cells. Thus, use of peptide mixes can allow flexibility in clinical research studies for which samples must be shipped or cryopreserved prior to analysis.

The complexity of peptide mixtures can be extensive, especially for large proteins, requiring 100 or more peptides to span the entire protein. To help facilitate the use of peptide mixes for CFC and other assays, BD Biosciences now offers peptide mixes for selected antigens through their custom program. Three such mixes, for CMV pp65, HIV p55 gag, and CEA are available in stock and can be ordered in small volumes. Examples of responses to these peptide mixes are shown in Figure 3.

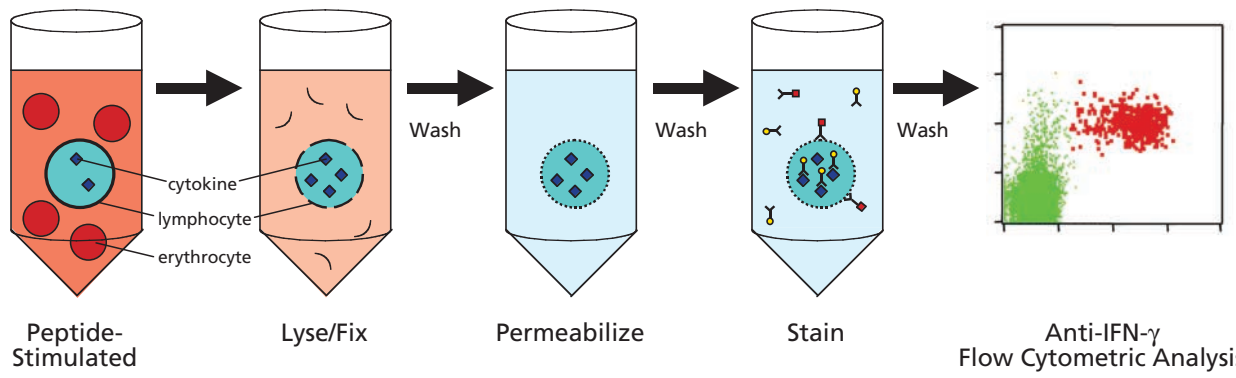


Figure 2. Overview of BD FastImmune assay for antigen-specific results in hours

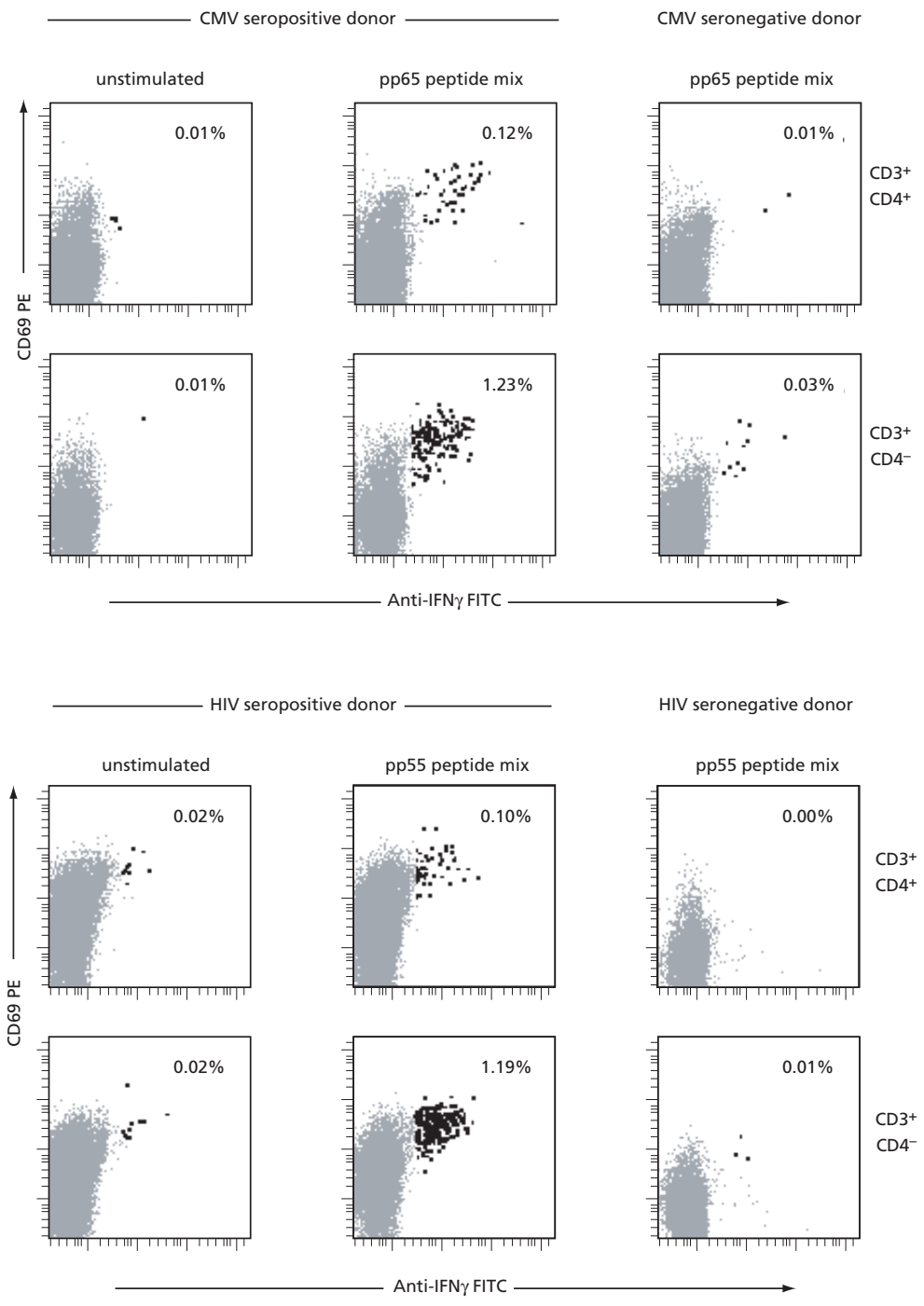


Figure 3. (A) Representative responses to CMV pp65 peptide mix in a CMV seropositive versus CMV seronegative donor (B) Representative responses to HIV p55 gag peptide mix in an HIV seropositive versus HIV seronegative donor

Custom Peptide Mixes for Cytokine Flow Cytometry (continued from page 13)

In conclusion, the use of peptide mixes of 15 mers overlapping by 11 amino acid residues appears to be efficient for detection of total CD4 and CD8 responses to an entire protein. Because the peptides are synthetically derived, they might offer more lot-to-lot stability than recombinant proteins or whole virus preparations. In addition, they appear optimally compatible with cryopreserved PBMCs or shipped whole blood. Thus, clinical research studies evaluating immunogenicity of vaccine preparations might benefit from use of such peptide mixes and CFC analysis.

Supporting Literature and Workshops

Please contact your local BD Biosciences representative for more detailed information.

- BD FastImmune CFC Handbook
  Performance Characteristics of Antigen-Specific Cytokine Flow Cytometry
- BD FastImmune Application Notes
  - Cytokine Detection in Antigen-Activated $CD8^+$ and $CD4^+$ T cells
  - Simultaneous Detection of Proliferation and Cytokine Expression in Peripheral Blood Mononuclear Cells
- Intracellular Cytokine Brochure
  Complementary brochure featuring other BD FastImmune intracellular reagents and protocols
- Intracellular Cytokine Workshop

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Custom Peptide Mixes & Cytokine Kits Product List

AVAILABLE CUSTOM PEPTIDE MIXES	SIZE	CAT. NO.	PRICE
CMV pp65 peptide mix (138 Peptides)	5 Test[a]	**551969**	$125
HIV p55 gag peptide mix (127 Peptides)	5 Test[a]	**551940**	$125
CEA peptide mix (173 Peptides)	5 Test[a]	**552821**	$125

[a] Each test is sufficient for activation of 1 mL whole blood or 1 mL PBMC at 1 × 10^6 cells/mL.

BD FASTIMMUNE CYTOKINE SYSTEM REAGENTS	FORMAT	SIZE	CAT. NO.	PRICE
BD FastImmune CD8 Anti-Hu–IFN-γ Intracellular Detection Kit	FITC/PE/PerCP-Cy5.5/APC	25 tests	**346049***	$995
BD FastImmune CD4 Anti-Hu–IFN-γ Intracellular Detection Kit	FITC/PE/PerCP-Cy5.5	25 tests	**340970***	$895
BD FastImmune CD4 Anti-Hu–IL-2 Intracellular Detection Kit	FITC/PE/PerCP-Cy5.5	25 tests	**340971***	$895
BD FastImmune CD4 Anti-Hu–TNF-α Intracellular Detection Kit	FITC/PE/PerCP-Cy5.5	25 tests	**340972***	$895
BD FastImmune Anti-Hu–IFN-γ /CD69/CD8/CD3	FITC/PE/PerCP-Cy5.5/APC	50 tests	**346047***	$650
BD FastImmune Isotype Control IgG2a/IgG1/CD8/CD3	FITC/PE/PerCP-Cy5.5/APC	50 tests	**346048**	$445
BD FastImmune Anti-Hu–IFN-γ/CD69/CD4	FITC/PE/PerCP-Cy5.5	50 tests	**340962***	$620
BD FastImmune Anti-Hu–IL-2/CD69/CD4	FITC/PE/PerCP-Cy5.5	50 tests	**340963***	$620
BD FastImmune Anti-Hu–TNF-γ/CD69/CD4	FITC/PE/PerCP-Cy5.5	50 tests	**340964***	$620
BD FastImmune Isotype Control IgG2a/IgG1/CD4	FITC/PE/PerCP-Cy5.5	50 tests	**340965**	$415
Use During Sample Activation				
BD FastImmune CD28/CD49d Costimulatory Reagent, 1×[a]		300 mL	**347690**	$135
BD FastImmune Brefeldin A Solution, 10×[b]		250 mL	**347688**	$135
BD FastImmune EDTA Solution, 1×[c]		2.50 mL	**347689**	$75
Use for Lysis and Permeabilization Post Sample Activation				
BD FACS Lysing Solution, 10× (150 tests standard protocol)		30 mL	**347691**	$105
BD FACS Lysing Solution, 10× (500 tests standard protocol)		100 mL	**349202**	$345
BD FACS Permeabilizing Solution 2, 10× (200 tests)		10 mL	**347692**	$170
BD FACS Permeabilizing Solution 2, 10× (500 tests)		25 mL	**340973**	$430

[a] Use at 5 mL/0.5 mL whole blood.
[b] Dilute 1:10 with sterile PBS and use at 1× concentration, 10 mL/0.5 mL of whole blood.
[c] Use at 1× concentration at 50 mL/0.5 mL of whole blood.

All products in this table are manufactured under cGMP

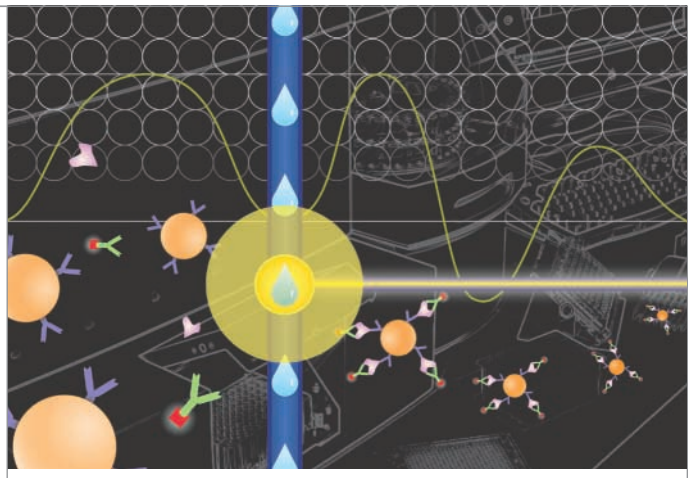
* Use of these products to measure activation antigens expressed on mononuclear cell subsets for purpose of monitoring immunoregulatory status can fall under one or more claims of the following patents: US 5,445,939; 5,656,446; 5,843,689; Europe 319,543; Canada 1,296,622; Australia 615,880; and Japan 2,769,156.

† Patents—PE and APC: US 4,520,110; 4,859,582; 5,055,556; Europe 76,695; Canada 1,179,942 PerCP: US 4,876,190 Cy5.5: US 5,268,486; 5,486,616; 5,569,587; 5,569,766; 5,627,027
BD FACS Lysing Solution: US 4,654,312; 4,902,613; 5,098,849

BD™ Cytometric Bead Array Program Update

BD Biosciences Pharmingen is pleased to announce two new BD™ Cytometric Bead Array (CBA) kits available in a pre-release format from the Custom Products and Services Group. A pre-release kit is a BD CBA Kit product available for only a limited time to researchers who want to try new BD CBA products before they are widely available off-the-shelf. Pre-release BD CBA Kits offered by the Custom Products and Services Group have a different part number than the regular BD CBA Kit of the same name which will be available in the near future. There is no difference in kit performance between Pre-release BD CBA Kits and our regular BD CBA Kits – they are just available sooner.

Currently available as Pre-release BD CBA Kits are the BD CBA Human Anaphylatoxin Kit and the BD CBA Mouse Inflammation Kit. The BD CBA Human Anaphylatoxin Kit quantitatively measures the human complement proteins C4a, C3a, and C5a (bioactive cleavage products released from C4, C3, and C5 respectively during complement activation) in serum or plasma samples. The BD CBA Mouse Inflammation Kit quantitatively measures levels of mouse Interleukin-6 (IL-6),



Interleukin-10 (IL-10), Monocyte Chemoattractant Protein-1 (MCP-1), Interferon-γ (IFN-γ), Tumor Necrosis Factor-α (TNF-α), and Interleukin-12p70 (IL-12p70) in tissue culture supernatant, serum, or EDTA plasma samples.

Contact the Custom Products and Services Group (**858.812.8982 in the US**) directly for more information regarding the availability of Pre-release CBA Kit products.

BD Cytometric Bead Array Kits Currently Available

DESCRIPTION	CONTENTS	SIZE	CAT. NO.
Human Kits			
Human Th1/Th2 Cytokine CBA Kit	IL-2, IL-4, IL-5, IL-10, TNF-α, IFN-γ	50 tests	**550749**
Human Th1/Th2 Cytokine CBA Kit - II	IL-2, IL-4, IL-6, IL-10, TNF-α, IFN-γ	50 tests	**551809**
Human Inflammation CBA Kit	IL-8, IL-1β, IL-6, IL-10, TNF-α, and IL-12p70	50 tests	**551811**
Human Active Caspase-3 CBA Kit	Active Caspase-3	100 tests	**552124**
Mouse Kits			
Mouse Th1/Th2 Cytokine CBA Kit	IL-2, IL-4, IL-5, TNF-α, IFN-γ	50 tests	**551287**
Mouse Immunoglobulin Isotyping CBA Kit	Heavy and light chain isotypes of mouse IgG1, IgG2a , IgG2b ,IgG3 , IgA, IgM, IgE	100 tests	**550026**
Other			
Human Th1/Th2 Cytokine Standards	IL-2, IL-4, IL-5, IL-6, IL-10, TNF-α, IFN-γ, lyophilized	1 vial	**551810**
BD CBA Software	Mac and PC Compatible CD-Rom and User's guide	1 CD	**550065**

BD Cytometric Bead Array Kits Available from the Custom Products and Services Group

DESCRIPTION	CONTENTS	SIZE	CUSTOM CAT. NO.
Mouse Inflammation CBA Kit	Mouse IL-6, IL-10, MCP-1, IFN-γ, TNF-α, IL-12p70	50 tests	**552563**
Human Anaphylatoxin CBA Kit	Human C4a, C3a, C5a	50 tests	**552690**

Table 1. BD CBA Software Matrix

	Version 1.1	Version 1.2	Version 1.3 Mac & PC
Excel 98	x		x
Excel 2000 (PC only)		x	x
Excel 2001	x		x
Excel v.X for OS X			x
OS 8.1 to OS X	x		x
Windows 98		x	x
Window NT 4.10 & 2000		x	x
Macintosh G3/G4	x		x
PC		x	x
Int'l Mac OS versions	x		x
Variable parameter map			x
Sample replicate calculation			x

New enhancements to the BD CBA Software – Version 1.3

The BD CBA analysis software is a powerful and flexible tool built as an Add-In for Microsoft Excel 98, 2001, and version X on the Macintosh PowerPC, G3, or G4 computer or Excel 2000 for Microsoft Windows (Table 1). Accommodating multiple sizes and intensities of beads, it provides a foundation for bead array analyses in the research environment. A wide variety of preset configurations enable the user to set up standard dilution series, generate calibration curves, and subsequently compare unknowns. Appropriate reports can be generated from each step in the process. The analysis of BD CBA data is optimized when using the BD CBA Software.

New features in the BD CBA software version 1.3 include variable parameter mapping to facilitate the analysis of files from a variety of flow cytometers. Version 1.3 also enables the determination of mean, standard deviation, and % c.v. statistics for multiple sample replicates, use of standard concentration values between 0.0001 and 1.0, and dialog enhancements. The software should be installed according to the instructions in the Software User's Guide.

NEW PE-Cy7 Tandem Conjugates for Mouse Research

By Fariba Hosseini, Tiffany Clarke, Belen Ybarrondo

Instrumentation and Background

PE-Cy7 is a tandem fluorochrome consisting of R-phycoerythrin (PE), a protein which is excited by an Argon laser at 488 nm and acts as an energy donor, coupled to the cyanine dye Cy7, a small organic molecule which is an energy acceptor and emits at 767 nm. The PE-Cy7 tandem conjugate emission is detected in the FL3 channel of BD FACScan™ and BD FACSCalibur™ flow cytometers and can also be detected using the BD LSR and the BD FACSVantage™ flow cytometry systems.

Advantages

PE-Cy7 conjugated reagents are as bright as PE conjugates (Figure 1) and can be used simultaneously with other fluorochromes such as FITC, PE, Cy-Chrome, PerCP, PerCP-Cy5.5, PE-Texas Red, APC, and APC-Cy7 with minimal compensation, making PE-Cy7 conjugates very useful for four, five, and six color applications.

New Products

PE-Cy7 conjugated antibodies recently have been added to the growing list of BD Biosciences fluorochrome conjugates. BD Biosciences Pharmingen now offers PE-Cy7 conjugated anti-mouse antibodies with a high standard of quality, low background, bright signal, and low cross over (Figure 2).

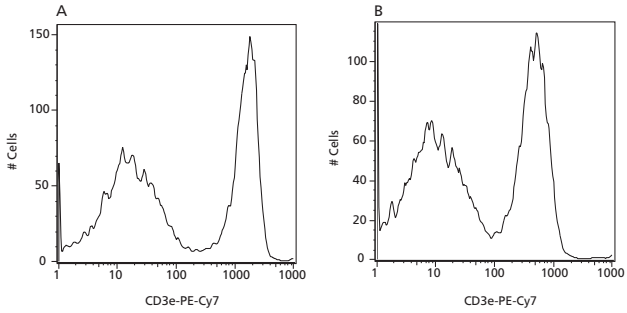
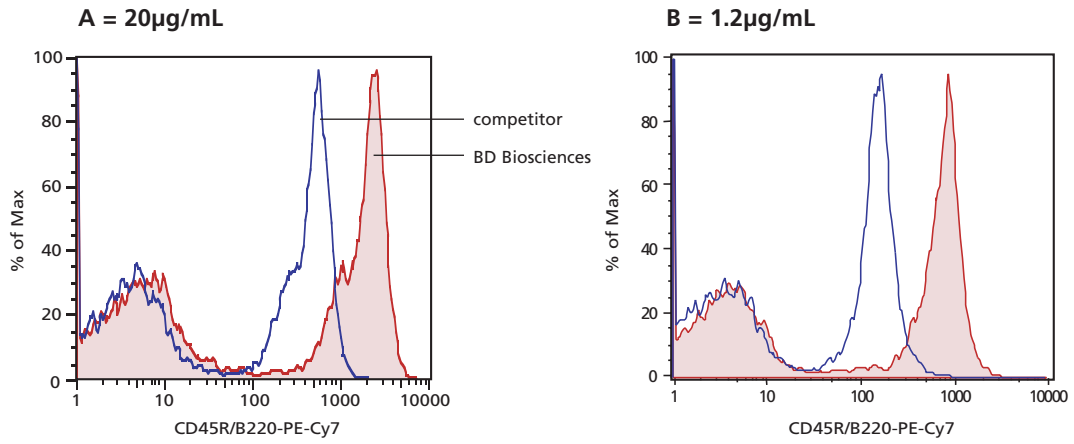


Figure 1. Comparison of anti-CD3e PE-Cy7 and PE Conjugates.

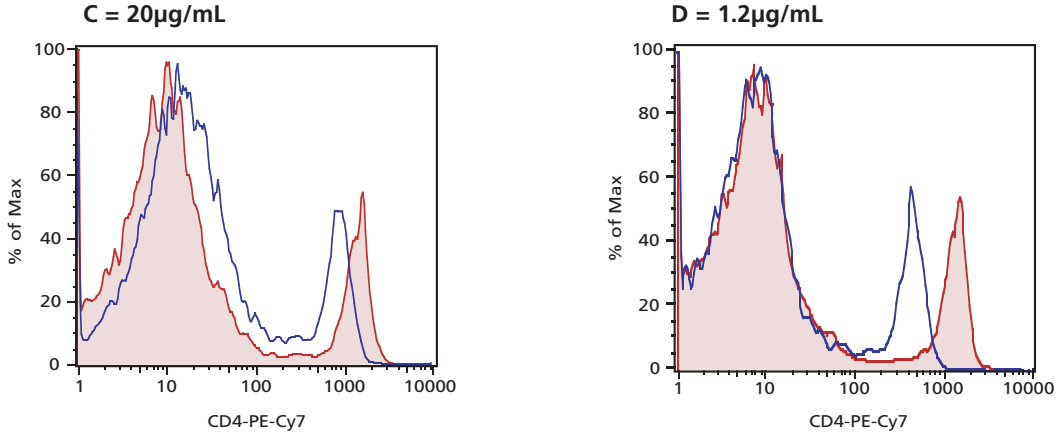
Mouse spleen cells were stained for cell surface expression of CD3e (clone 145-2C11), using either PE-Cy7 (Figure A) or PE (Figure B). Flow cytometry was performed on the BD FACSCalibur™ (BD Biosciences, San Jose, CA)

Mouse PE-Cy7 Antibody Reagents from BD Biosciences Pharmingen

DESCRIPTION	CLONE	SIZE	CAT. NO.
CD3e	145-2C11	0.1mg	552774
CD4	RM4-5	0.1mg	552775
CD8a	53-6.7	0.1mg	inquire
CD11b	M1/70	0.1mg	552850
CD19	1D3	0.1mg	552854
CD25	PC61	0.1mg	inquire
CD45	30-F11	0.1mg	552848
CD45R/B220	RA3-6B2	0.1mg	552772
CD69	H1.2F3	0.1mg	inquire
NK1.1	PK136	0.1mg	inquire
IgM	R6-60	0.1mg	inquire
Hamster IgG1, k Isotype Control	A19-3	0.1mg	552811
Mouse IgG2a, k Isotype Control	G155-178	0.1mg	inquire
Rat IgG1,lambda Isotype Control	A110-1	0.1mg	inquire
Rat IgG2a, k Isotype Control	R35-95	0.1mg	552784
Rat IgG2b, k Isotype Control	A95-1	0.1mg	552849



	FL3 Median	Cross-over Into FL2 Channel	FL3 Median	Cross-Over Into FL2 Channel
BD Biosciences (shaded)	1994	5.2	813	4.8
Competitor (open)	491	9.5	160	9.9



	FL3 Median	Cross-over Into FL2 Channel	FL3 Median	Cross-Over Into FL2 Channel
BD Biosciences (shaded)	1369	5.3	1321	5.6
Competitor (open)	778	8.8	739	9.7

Figure 2. Anti-CD45R/B220 and anti-CD4 PE-Cy7 Compared to equivalent Competitor Reagent

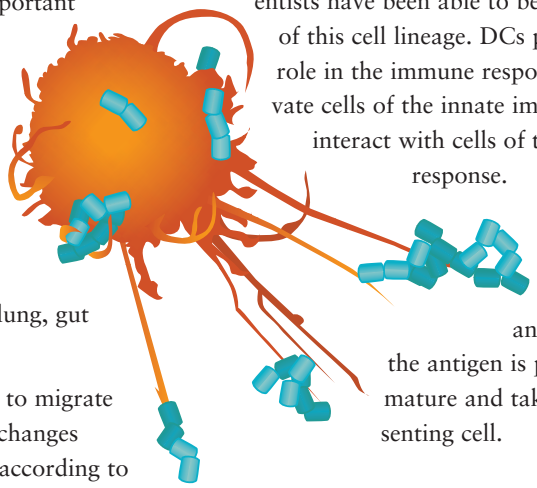
Mouse spleen cells were stained for cell surface expression of CD45R/B220 (A and B) or CD4 (C and D) using either BD Biosciences PE-Cy7 reagents (shaded (red) histogram) or a competitor's reagent (overlay (blue) histogram) at 20µg/mL (Figure A and C) and at 1.2µg/mL (Figure B and D). Flow cytometry was performed on the BD FACSCalibur (BD Biosciences, San Jose, CA). Percent cross-over is defined as the percent of PE-Cy7 fluorescence that is detected in the FL2 channel in an uncompensated sample.

Reagents for Human Dendritic Cell Research

By Enoc Hollemweguer, David J. Eling, and Li Li

Dendritic cells (DCs) are known as the professional antigen presenting cells. DCs perform the important role of capturing and processing antigen and presenting it to naïve T-helper cells to initiate the immune response. Distinct subsets of DCs have been described. They include interdigitating reticulum cells in lymphoid organs, blood DCs, Langerhans cells and dermal DCs when found in the dermis. Dendritic cells are also present on other non-lymphoid organs including heart, lung, gut and synovium.

DCs show great heterogeneity. They are able to migrate through various tissues and their phenotype changes according to their stage of differentiation or according to their response and interaction with cytokines, chemokines and/or other growth factors in their microenvironment. They present a scientific challenge because there are very few monoclonal antibody that specifically recognize all DCs. With the use of various monoclonal antibodies, scientists have been able to better understand the biology of this cell lineage. DCs play a unique and important role in the immune response, as they recruit and activate cells of the innate immune response as well as interact with cells of the specific immune response.



Studies show that the immature DCs have the greatest phagocytic or antigen capture capacity. As the antigen is processed, DCs continue to mature and take the role of antigen presenting cell.

Antibodies to Dendritic Cell Associated Antigens from BD Biosciences Pharmingen

DESCRIPTION	CLONE	EXPRESSION
CD123	9F5	Lymphoid DC Precursor
CD11c	B-ly6	Myeloid DC Precursor
CCR5	2D7/CCR5	Immature DC
CCR6	11A9	Immature DC
CD68	Y1/82A	Immature DC
CD206 (Mannose Receptor)	19	Immature DC
CD36	CB38	Immature DC
CD51/CD61	23C6	Immature DC
CD47	B6H12	Immature DC
CD87	VIM5	Immature DC
CD95	DX2, EOS.9	Immature DC
CD209 (DC-SIGN)	DCN46	Immature DC
TAP1	TAP1.28	Antigen Processing
TAP2	TAP2.17	Antigen Processing
HLA-DM	MAP.DM	Antigen Processing
CLIP	CerCLIP	Antigen Processing
CCR7	2H4, 3D12	Mature DC
CXCR4 (fusin)	12G5	Mature DC
CD205 (DEC-205)	MG38	Mature DC
CD83	HB15e	Mature DC
CD1a	HI149	Antigen Presentation
CD1b	M-T101	Antigen Presentation

DESCRIPTION	CLONE	EXPRESSION
CD1c	11.86	Antigen Presentation
CD1d	CD1d42	Antigen Presentation
HLA-A,B,C	G46-2.6	Antigen Presentation
HLA-DR	G46-6	Antigen Presentation
CD80	L307.4	Mature DC
CD86	FUN-1, IT2.2	Mature DC
CD40	5C3	Mature DC
CMRF-44	CMRF-44	Mature DC
CMRF-56	CMRF-56	Mature DC
B7-H2	2D3/B7-H2	Immature DC

*Visit **www.bdbiosciences.com** for product details and technical datasheets.*

NEW CFSE T-cell Proliferation Kit

By Enoc Hollemweguer, Jeanne Elia, David Ernst, and Carol Guenther

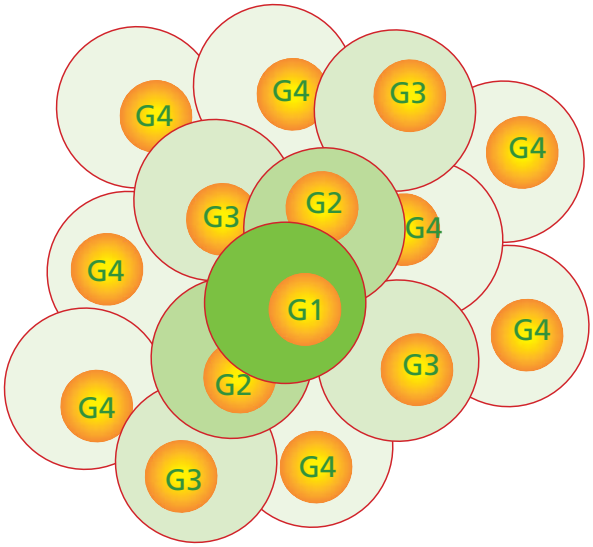


Figure 1. Upon cell division, CFSE is distributed uniformly between daughter cells.

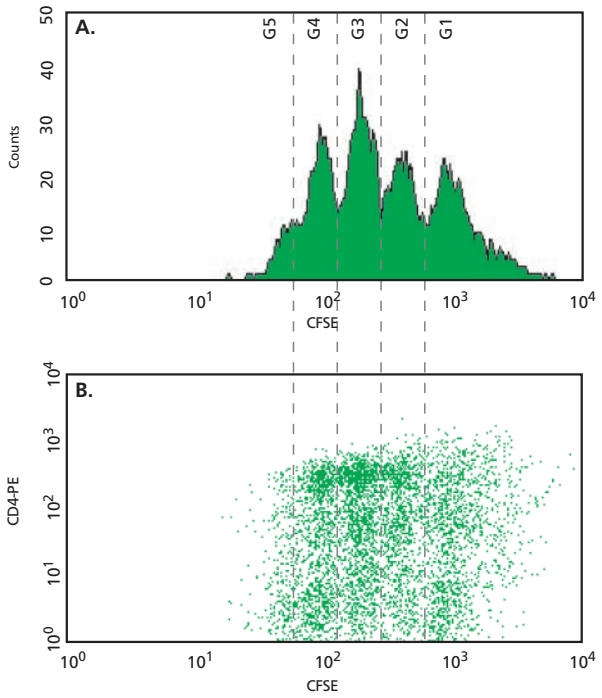


Figure 2. Profile of CSFE-labeled, PHA-stimulated (72 hrs), peripheral blood mononuclear cells (PBMCs) analyzed by flow cytometry.

Carboxy-fluoresceindiacetate succinimidyl ester (CFDA SE) is a very effective reagent for studying and following the division progress of proliferating cells. It passively crosses the cell membrane and covalently binds to free amine groups of intracellular macromolecules. Endogenous cytoplasmic esterases remove the carboxyl groups, converting non-fluorescent CFDA SE to fluorescent CFSE. Upon cell division, CFSE is distributed uniformly between daughter cells (Figure 1). Each cell division reduces the CFSE fluorescent intensity of daughter cells by half in flow cytometric analysis. The multipeak histogram (Figure 2A) shows several divisions that human peripheral blood lymphocytes have undergone when cultured for 72 hr with phytohemaglutinin.

By using CFSE as a dye for tracking cell proliferation, one can select additional parameters (eg, CD markers or intracellular cytokines) and perform further flow cytometric analysis to characterize the nature of cells within any cell generation. For example, as shown in Figure 2B, CFSE staining can be coupled with staining for cell surface CD4 to identify the proliferative profiles of $CD4^+$ cells. This offers a definite advantage over other methodologies like [^{3}H]-thymidine incorporation and MTT that provide information concerning the growth or S-phase activity of cells at the cell population level.

Coming Soon from BD Biosciences Pharmingen is the CFSE T-cell Proliferation Kit which contains the reagents and instructions necessary to easily and reproducibly perform cell proliferation experiments. The protocol has been used on human cells, but it also works well with mouse cells. This kit addresses the problem of excess CFSE concentrations leading to overly bright fluorescent intensity and poor separation of the peaks (generations). All reagents, including the CFSE solution as well as antibodies to identify cell subpopulations, have been optimized for use with human peripheral blood mononuclear cells (PBMCs) when following the volume recommendations included in the kit, distinct, separate peaks for each generation should be easily detected (Figure 2).

NEW Non-human Primate CD45 Reagent

By Xiao-wei Wu, Glenn Krasinki,
and Enoc Hollemweguer

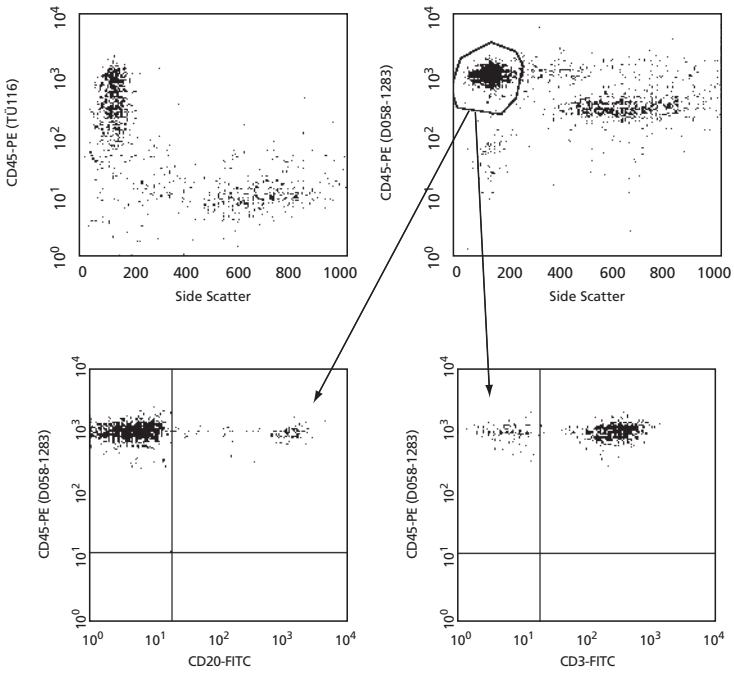


Figure 1. Profile of anti-CD20 (clone 2H7) and anti-CD3 (clone SP34-2) on anti-CD45 (clone D058-1283)-gated peripheral blood lymphocytes of rhesus macaque (Macaca mulatta) analyzed by flow cytometry.

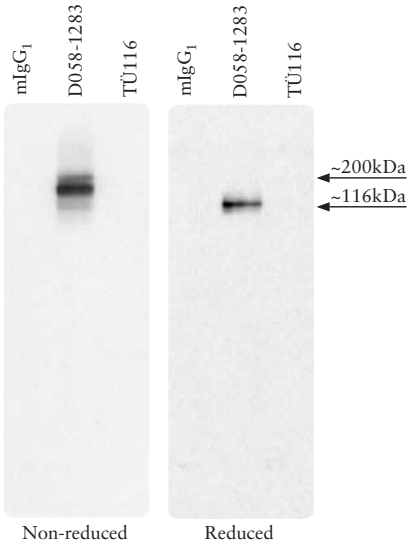


Figure 2. Western Blot analysis on peripheral blood of rhesus macaque (Macaca mulatta). Non-reduced sample shown at 1 second exposure, reduced sample shown at 10 seconds exposure.

BD Biosciences is pleased to announce D058-1283, a CD45 monoclonal antibody specific for non-human primate leukocytes, developed using rhesus peripheral whole blood as immunogen. It does not cross-react with human leukocytes. This antibody reacts with baboon, rhesus, and cynomolgus macaque leukocytes in a manner similar to CD45 binding of the Leukocyte Common Antigen on human cells. Immunophenotypic analysis shows that D058-1283 binds to lymphocytes, monocytes, and granulocytes of non-human primate blood samples (Figures 1 and 2). This antibody is able to block the binding of monoclonal antibody TÜ116, an anti-human CD45 antibody reported to cross-react with non-human primate leukocytes.

In western blot analysis D058-1283 yeilds a band of an approximate molecular weight of 180-200 kDa. The data shown demonstrates its utility as a gating tool for non-human primate flow cytometric analysis.

References:

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3. Reimann K A, Waite BCD, Lee-Parritz DE, et al. Use of human leukocyte-specific monoclonal antibodies for clinically immunophenotyping lymphocytes of rhesus monkeys. Cytometry. 1994;17:10

Mouse Cell Activation

By Ravi Hingorani

In response to invading pathogens, the host mounts a vigorous attack through its immune system. This response, at various levels, involves either direct or indirect stimulation of T cells, B cells, neutrophils, and macrophages. Stimulation can be a direct event, as in the case of antigen presentation to specific populations of cells, or an indirect event as in a response to soluble factors such as IL-2, IL-4, IFN-γ, et cet. The complexity of the entire system is enormous when one considers the extensive interaction between different cell types. Within the complex immune network the T cell dependent responses have been the subject of intense investigation. In an effort to simplify such complex *in vivo* mechanisms, experimental *in vitro* model systems have often been used to study these events.

Studies on *in vitro* activation of T cells, in most cases, involve stimulation with antibodies to the anti-CD3 complex, or T cell receptor (TCR) α/β chains or with mitogens such as Concanavalin A (Con A), Phorbol Myristol Acetate (PMA) and Ionomycin. Different laboratories, according to their needs and familiarity with the assay systems, set up distinct culture conditions for *in vitro* stimulation. Different systems give rise to varying levels of *in vitro* activation that in turn can lead to different expression kinetics of activation-induced cell surface markers. Shown below are the changes in a few select activation markers on the $CD4^+$ and $CD8^+$ cells in response to stimulation via the CD3 complex. Multiparameter measurements using antibodies conjugated to different fluorochromes were carried out to detect the various subsets.

Protocol

Peripheral lymph node (LN) cells were collected from BALB/c (6-10 weeks old) mice and incubated at 37°C for 1, 2, or 3 days with immobilized anti-CD3e (clone 145-2C11. After culturing cells for different periods of time, cells were stained with PE-conjugated antibodies specific for selected cell-surface activation markers. The T-cell subsets were identified by staining with FITC conjugated anti-CD8 and APC conjugated anti-CD4, while the dead cells were discriminated by using propidium iodide (PI). Flow cytometry was performed on the BD FACSCalibur™ Flow Cytometry System and analysis of the activation markers was carried out using the BD CellQuest™ Analysis program.

In an effort to reproducibly measure fluorescence (and thus receptor) densities, the PMT voltages on the BD FACSCalibur were standardized for every experiment using Rainbow Fluorescent Calibration particles (Cat. No. 556298). In all experiments, samples with PE conjugated isotype controls were also analyzed. Staining with control antibodies did not result in any shift in their fluorescence intensity (data not shown).

An example of the analysis profile with detailed gating regions is given in Figure 1. A lymphocyte gate based on the forward vs. side scatter profile (R1) was used to obtain the PI profile on different days (Figures 1A and B). As expected, enhanced cell death due to the turnover of actively cycling cells is associated with stimulation over time (seen as an increase in the PI positive fraction). Gating out dead cells is important since they non-specifically bind to antibodies and thus show up as false positives. The PI negative fraction containing the viable cell fraction (R2) was used for subsequent analysis of the CD4 and CD8 populations (Figure 1C). Finally, the CD4 (R3) and CD8 (R4) positive populations were analyzed for the intensity of the activation-induced markers.

Mouse Cell Activation (continued from page 23)

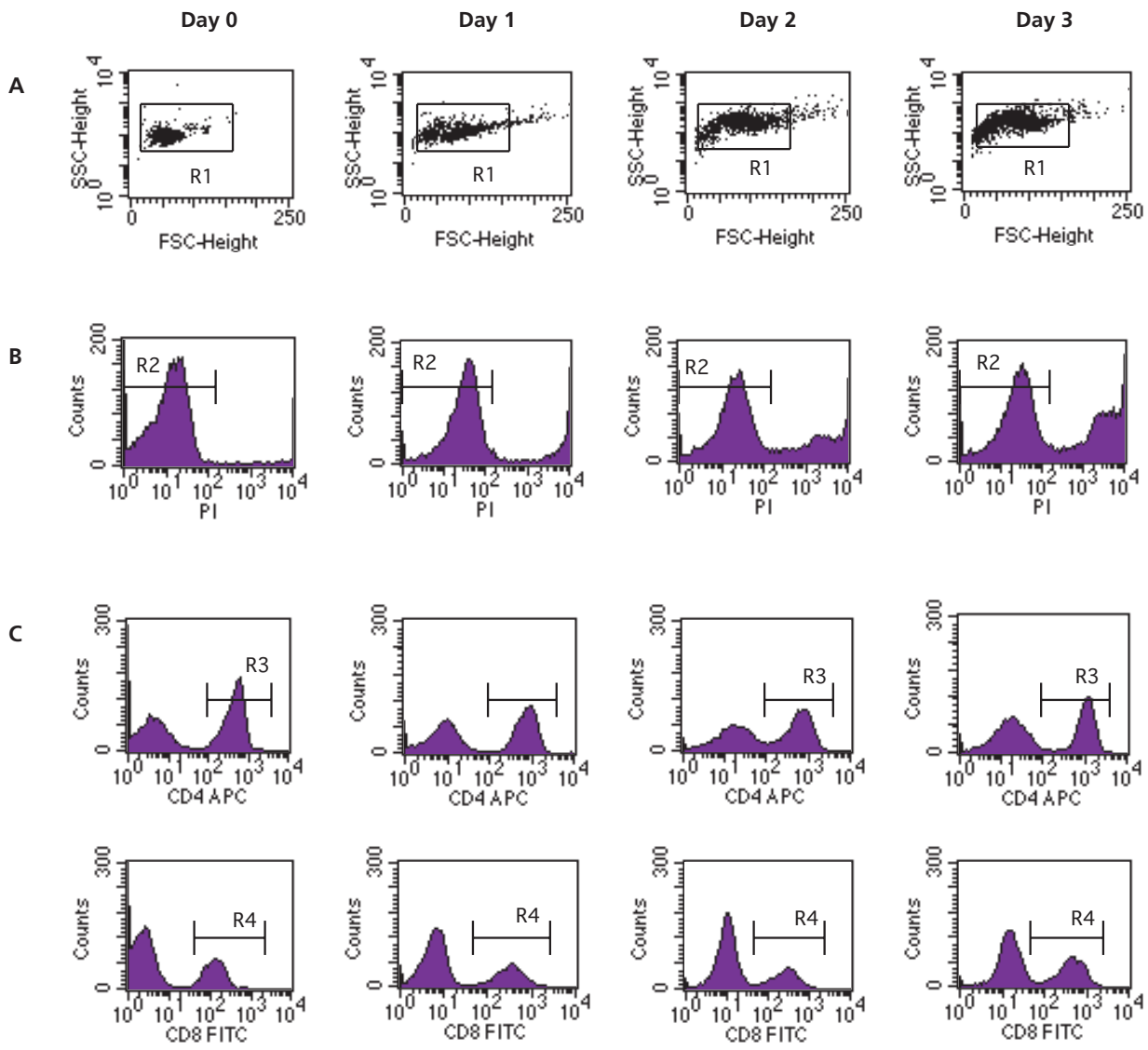

Figure 1. Analysis profiles of staining at day 0, 1, 2, and 3. Cells incubated with plate-bound anti-CD3e for different periods of time were analyzed as shown above.

αβ TCR and co-receptors

Only slight differences were observed between the profiles of CD4 and CD8 cells for the αβ TCR receptor (Figure 2). There was a decrease in the TCR levels seen on the CD4 subset that returned back to initial levels on day 2 and remained at comparable levels till the end of the culture at day 3. No such decrease in TCR levels in the CD8 subset was seen on day 1. However, on the final day, day 3, a slight increase in the TCR expression was observed.

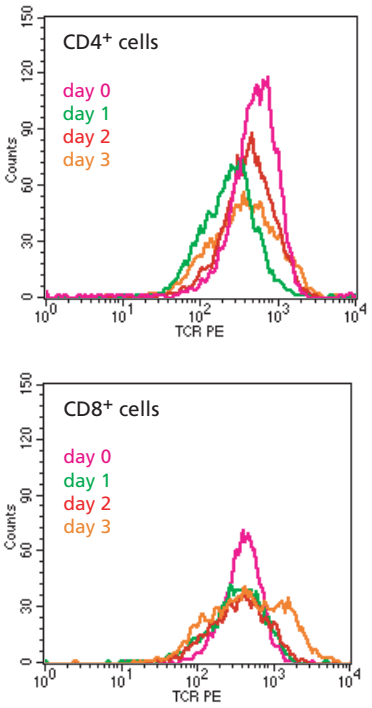

Figure 2

CD11a (Integrin α_L chain)

There was an increase of CD11a on the CD4 and CD8 cells after 24h stimulation with anti-CD3 (Figure 3). The highest expression was observed on day 3 on both the $CD4^+$ and the $CD8^+$ T cell populations.

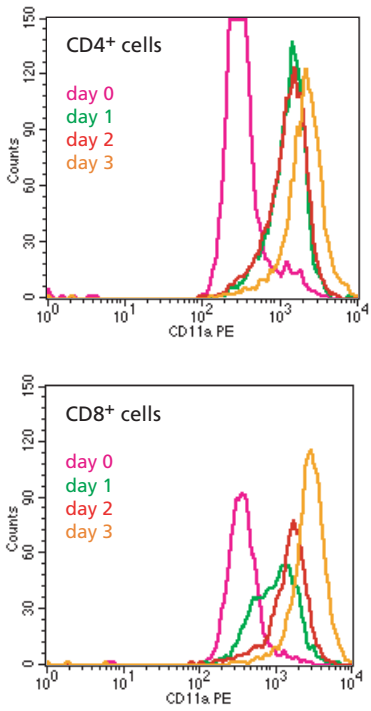

Figure 3

Mouse Cell Activation (continued from page 25)

CD25 (IL-2 Receptor α chain)

Activation via the CD3 complex up-regulates expression of CD25 on T cells, as is shown in the histogram below (Figure 4). Only a small percentage of CD25 positive cells was observed at the start of the stimulation, mainly in the CD4 population. However, a rapid induction of this receptor on all T cells was observed after 24h of anti-CD3e stimulation. Compared to the immediate increase on CD4 cells, the increase in the surface expression of CD25 on CD8 cells was more gradual. However, by day 3, both subsets of T cells showed high expression of CD25 with the CD8 cells showing a slightly higher intensity compared to the CD4 cells.

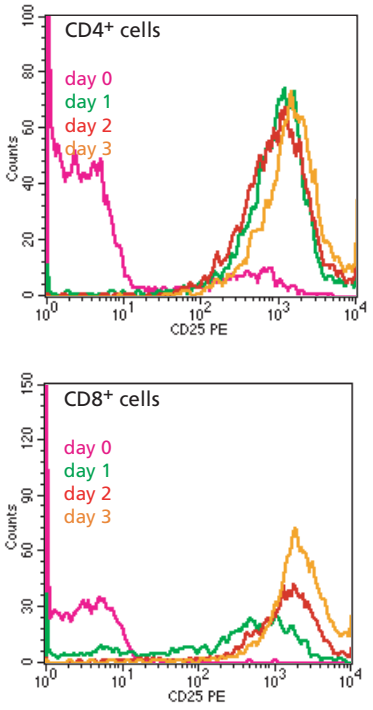

Figure 4

CD27

Up-regulation of CD27 following anti-CD3e stimulation was similar for CD4 and CD8 T cells (Figure 5). The relative increase in both the subsets was similar, with the maximal expression seen on day 3.

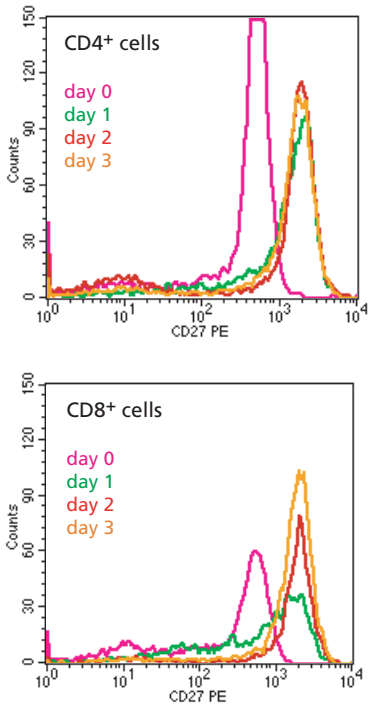

Figure 5

CD28

The up-regulation of CD28 expression on CD8 cells was more gradual than that of the CD4 cells (Figure 6). By day 3, both subsets showed similar levels of expression.

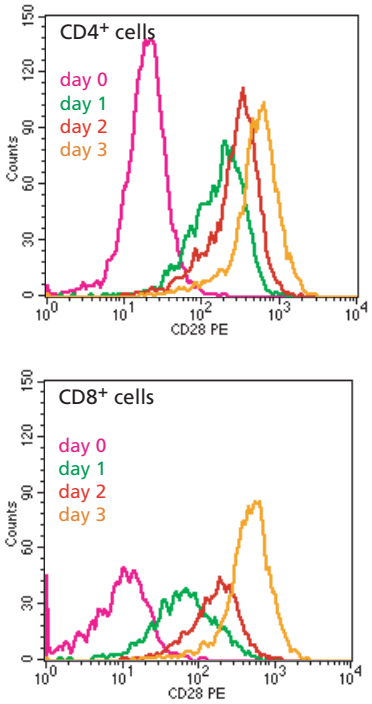

Figure 6

CD44 (Ly-24)

The increase in expression of CD44 was similar on both CD4 and CD8 T cells (Figure 7), with maximal expression observed on day 3.

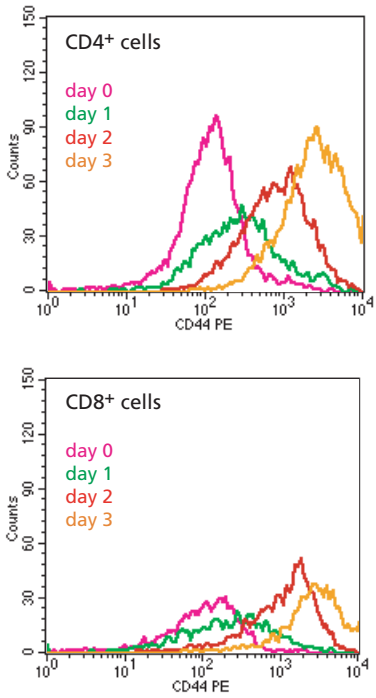

Figure 7

Mouse Cell Activation (continued from page 27)

CD49e (Integrin α_5 chain)

Under the *in vitro* conditions used, CD49e was rapidly expressed upon activation, with the highest expression observed on day 2 following anti-CD3 stimulation. Following this increase, a slight decrease was observed on both CD4 and CD8 populations on day 3 (Figure 8).

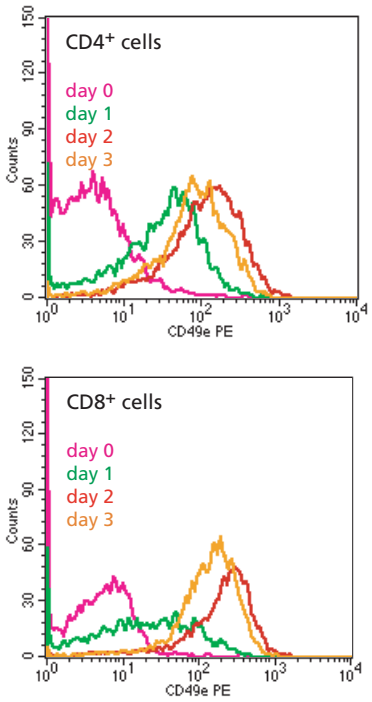

Figure 8

CD62L (L-selectin)

Both the CD4 and CD8 populations showed a transient decrease in CD62L expression after 24h of activation (Figure 9). Though the expression levels on day 2 and 3 for CD4 cells returned to initial levels, expression on CD8 cells on day 3 showed another decrease.

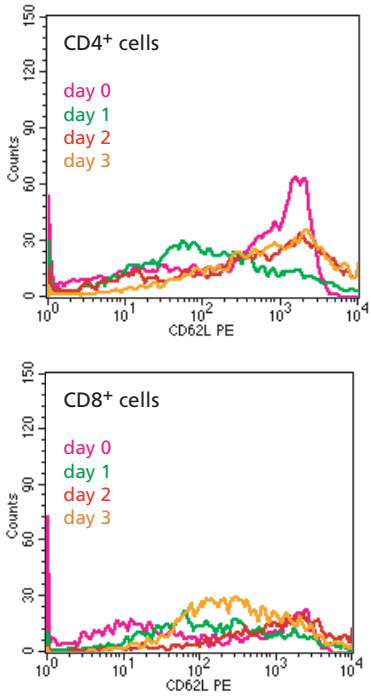

Figure 9

CD69 (Very Early Activation antigen)

Resting or naïve T cells express very little CD69 on the cell surface, as is shown in Figure 10. Upon anti-CD3e stimulation, a rapid induction of the expression was observed after 24h on the CD4 and CD8 populations. Subsequently, a decrease in the expression was observed on day 2, for both the CD4 and CD8 populations. Finally on day 3 both sub-populations again showed an increase in the expression, with a higher increase in the expression on the CD8 population than on the CD4 population.

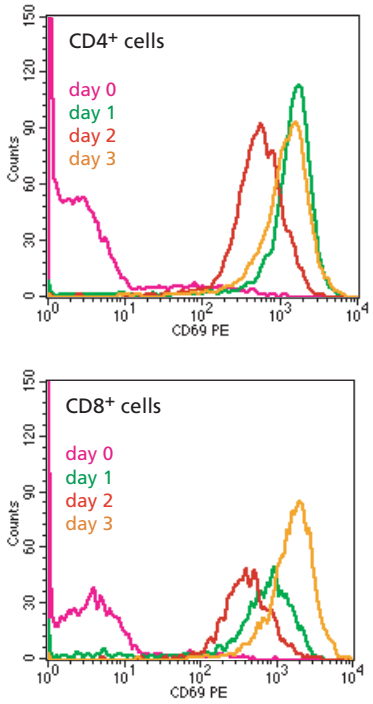

Figure 10

CD71 (Transferrin Receptor)

As can be seen in Figure 11 there was a rapid induction of CD71 upon activation with anti-CD3. The highest induction of CD71 was observed on day 2 of anti-CD3 activation on both CD4 and CD8 T cells. The intensity of CD71 then decreased slightly on day 3.

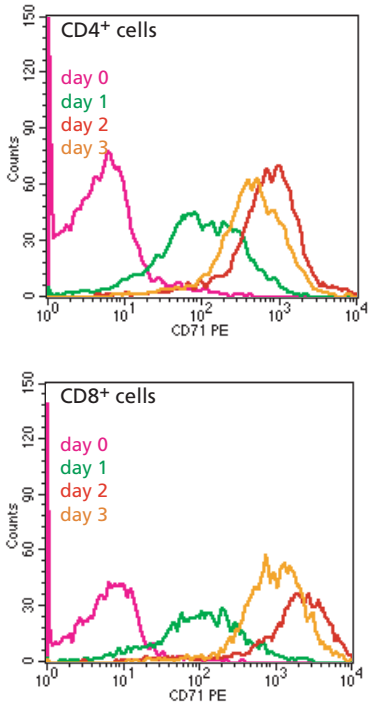

Figure 11

Mouse Cell Activation (continued from page 29)

CD95 (FAS)

As can be seen in Figure 12, Fas expression under our culture conditions increased after stimulation. The highest expression was observed after three days of stimulation with anti-CD3e. A similar increase in intensity was observed in both the CD4 and CD8 populations.

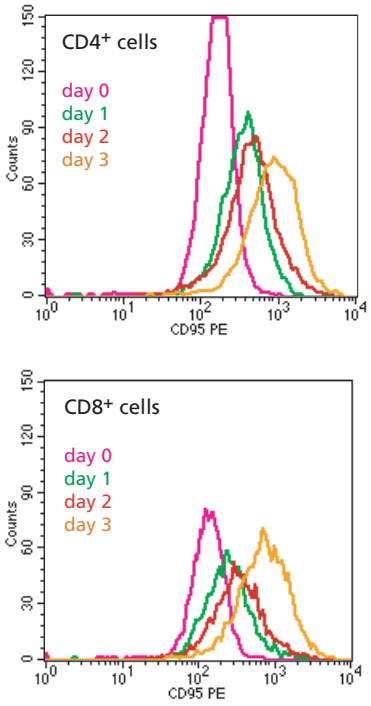

Figure 12

Ly-6A/E (Sca-1)

Upon activation with anti-CD3e, the expression of Ly-6A/E antigen increases on the CD4 and CD8 populations (Figure 13) with different kinetics. No expression was seen on CD4 cells at the start of the culture (day "0"), followed by intermediate level of expression after day 2. In contrast, low levels of expression seen at the start of the culture on CD8 cells increased to intermediate levels on day 1. By day 3 comparable expression of Ly-6A/E antigen was observed on both populations.

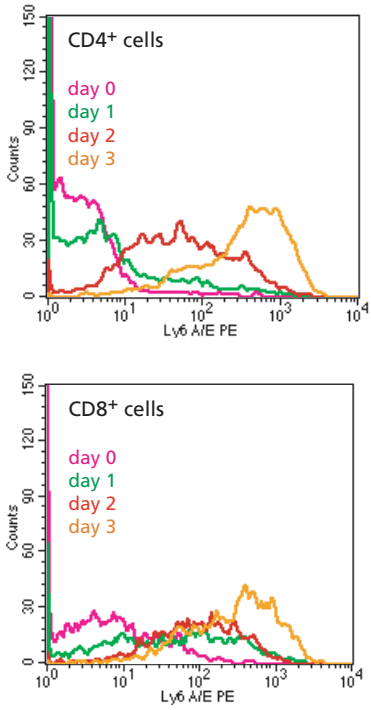

Figure 13

Mouse Antibodies for Cell Activation Research

ANTIGEN	CLONE
CD3	145-2C11
CD4	RM4-5
CD8	53-6.7
TCRβ	H57-597
CD11a	2D7
CD25	PC61
CD27	LG.3A10.1
CD28	37.51
CD44	IM7
CD49e	5H10-27
CD62L	MEL-14
CD69	H1.2F3
CD71	C2
CD95	Jo2
Ly6A/E	D7

For a complete listing of available formats of these and other activation-associated cell surface markers, please visit www.bdbiosciences.com

2003 Catalog Product Supplement for BD Biosciences

BD Biosciences Immunocytometry Systems

BD FastImmune Reagents

DESCRIPTION	REACT	CLONE	ISOTYPE	APPS	REG	FORMAT	SIZE	CAT. NO.	PRICE
BD FastImmune™ CD63/CD123/Anti–HLA-DR	Hu	H5C6, 9F5, L243		FCM	**RUO (GMP)***	FITC, PE, PerCP	50 tests	**341068**	$620

Viability Testing

DESCRIPTION	APPS	REG	FORMAT	SIZE	CAT. NO.	PRICE
BD™ Cell Viability Kit	FCM	**RUO (GMP)***	Thiazole Orange/ Propidium Iodide	100 tests	**349483**	$145
BD™ Cell Viability Kit with BD™ Liquid Counting Beads	FCM	**RUO (GMP)***	Thiazole Orange/ Propidium Iodide	100 tests	**349480**	$395

Human Cell Surface Molecules

DESCRIPTION	REACT	CLONE	ISOTYPE	APPS	REG	FORMAT	SIZE	CAT. NO.	PRICE
Anti-Kappa	Hu	TB 28-2	Mouse IgG_1	FCM	**RUO (GMP)***	APC	100 tests	**341088**	$510
CD3	Hu	SK7	Mouse IgG_1	FCM	**RUO (GMP)***	PE-Cy7	100 tests	**341091**	$550
CD3	Hu	SK7	Mouse IgG_1	FCM	**RUO (GMP)***	APC-Cy7	100 tests	**341090**	$550
CD4	Hu	SK3	Mouse IgG_1	FCM	**RUO (GMP)***	APC-Cy7	100 tests	**341095**	$550
CD10	Hu	HI10a	Mouse IgG_1	FCM	**RUO (GMP)***	PE-Cy7	100 tests	**341092**	$550
CD14	Hu	M?9	Mouse IgG_{2b}	FCM	**RUO (GMP)***	APC-Cy7	100 tests	**333945**	$550
CD19	Hu	SJ25C1	Mouse IgG_1	FCM	**RUO (GMP)***	PE-Cy7	100 tests	**341093**	$550
CD33	Hu	P67.6	Mouse IgG_1	FCM	**RUO (GMP)***	PE-Cy7	100 tests	**333946**	$550
CD55	Hu	IA10	Mouse IgG_{2a}	FCM	**RUO (GMP)***	PE	50 tests	**341030**	$265
CD117	Hu	104D2	Mouse IgG_1	FCM	**RUO (GMP)***	PerCP-Cy5.5	50 tests	**333944**	$310
CD117	Hu	104D2	Mouse IgG_1	FCM	**RUO (GMP)***	APC	100 tests	**341096**	$510
CD138	Hu	M15	Mouse IgG_1	FCM	**RUO (GMP)***	FITC	50 tests	**347191**	$235
CD138	Hu	M15	Mouse IgG_1	FCM	**RUO (GMP)***	PE	50 tests	**347192**	$265
CD138	Hu	M15	Mouse IgG_1	FCM	**RUO (GMP)***	PerCP-Cy5.5	50 tests	**341087**	$310
CD138	Hu	M15	Mouse IgG_1	FCM	**RUO (GMP)***	APC	100 tests	**347193**	$510

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Applicable Patents

PE and APC: US 4,520,110; 4,859,582; 5,055,556; Europe 76,695; Canada 1,179,942
PerCP: US 4,876,190
Cy: US 5,268,486; 5,486,616; 5,569,587; 5,569,766; 5,627,027
PE-Cy7: 4,542,104
APC-Cy7: US 5,714,386

BD Biosciences Pharmingen

Adhesion Molecules

DESCRIPTION	REACT	CLONE	ISOTYPE	APPS	FORMAT	SIZE	CAT. NO.	PRICE
CD134 (OX40)	Hu	ACT35	Mouse IgG_1, κ	FCM	Cy-Chrome	100 tests	**551500**	$295

Cytokines, Chemokines, and Inflammatory Mediators

Inflammatory mediators and their receptors - Complement and their receptors

DESCRIPTION	REACT	CLONE	ISOTYPE	APPS	FORMAT	SIZE	CAT. NO.	PRICE
CD21 (CR2)	Hu	1048	Mouse IgG_1, κ	FCM	Purified	0.1 mg	**552727**	$120

Inflammatory mediators and their receptors - Other inflammatory mediators and their receptors

DESCRIPTION	REACT	CLONE	ISOTYPE	APPS	FORMAT	SIZE	CAT. NO.	PRICE
Toll-like Receptor 4 (TLR4)	Hu	HTA125	Mouse IgG_1, κ	FCM	Biotin	100 tests	**551975**	$215

Cytokines and their receptors

DESCRIPTION	REACT	CLONE	ISOTYPE	APPS	FORMAT	SIZE	CAT. NO.	PRICE
IL-12 Receptor β_2	Ms	HAM10B9	Hamster IgG	FCM	Purified	0.1 mg	**552819**	$120

BD OptEIA™ ELISA Sets and Kits

Chemiluminescent - Human

DESCRIPTION	REACT	APPS	SIZE	CAT. NO.	PRICE
Human IFN-γ BD OptEIA ELISA CL Kit	Hu	ELISA	1 plates	**551501**	$450
Human IL-2 BD OptEIA ELISA CL Kit	Hu	ELISA	1 plates	**551794**	$450
Human TNF-α BD OptEIA ELISA CL Kit	Hu	ELISA	1 plates	**551502**	$450

Product Supplement (continued from page 33)

BD ELISPOT Reagents

Human

DESCRIPTION	REACT	APPS	SIZE	CAT. NO.	PRICE
Human Granzyme B ELISPOT Set	Hu	ELISPOT	10 plates	**552572**	$850
Human Granzyme B ELISPOT Kit	Hu	ELISPOT	2 plates	**inquire**	inquire

Mouse

DESCRIPTION	REACT	APPS	SIZE	CAT. NO.	PRICE
Mouse IL-6 ELISPOT Set	Ms	ELISPOT	10 plates	**552567**	$850

Rat

DESCRIPTION	REACT	APPS	SIZE	CAT. NO.	PRICE
Rat IL-4 ELISPOT Set	Rat	ELISPOT	10 plates	**552570**	$850

Cell Biology / Cell Signaling

DESCRIPTION	REACT	CLONE	ISOTYPE	APPS	FORMAT	SIZE	CAT. NO.	PRICE
ACT1	Hu, Rat	Poly 1340	Rabbit Ig	WB	Serum	100 µl	**552816**	$210
Cdc25C	Hu	TC113	Mouse IgG_1	IF, IP, WB	Purified	150 µg	**550921**	$295
Caspase-2		Poly1323	Rabbit Ig	WB	Serum	100 µl	**552786**	$210
Caspase-3		Poly1325	Rabbit Ig	WB	Serum	100 µl	**552785**	$210
Caspase-7		Poly 1327	Rabbit	WB	Serum	100 µl	**552815**	$210
Caspase-10		Poly 1331	Rabbit	WB	Serum	100 µl	**552810**	$210
E2		Poly1061	Rabbit Ig	WB	Serum	0.1 ml	**552735**	$210
c-IAP1	Hu	F30-2285	Mouse IgG_1, κ	WB	Purified	50 µg	**552782**	$175
c-IAP1	Hu	F30-2285	Mouse IgG_1, κ	WB	Purified	150 µg	**552783**	$295
PKD		Poly1336	Rabbit Ig	WB	Serum	100 µl	**552817**	$210
PKD		Poly1337	Rabbit Ig	WB	Serum	100 µl	**552818**	$210
p35		Poly 1332	Rabbit Ig	WB	Serum	100 µl	**552812**	$210
TLR3	Hu	F28-1707	Mouse IgG_1, κ	WB	Purified	50 µg	**552753**	$175
TLR3	Hu	F28-1707	Mouse IgG_1, κ	WB	Purified	150 µg	**552752**	$295
T Cell PTPase	Hu	Poly	Rabbit Ig	WB	Purified	100 µl	**552763**	$210
PC12 Cell Lysate				WB	Lysate	500 µg	**611454**	$85
SW-13 Cell Lysate				WB	Lysate	500 µg	**611475**	$85

Human Cell Surface Molecules

DESCRIPTION	REACT	CLONE	ISOTYPE	APPS	FORMAT	SIZE	CAT. NO.	PRICE
Basophils	Hu	Bsp-1	Mouse IgM, κ	FCM	Purified	0.1 mg	**552754**	$120
CD21 (CR2)	Hu	1048	Mouse IgG_1, κ	FCM	Purified	0.1 mg	**552727**	$120
CD66	Hu	COL-1	Mouse IgG_{2a}, κ	FCM	Purified	0.1 mg	**551477**	$120
CD66 (CEA, carcinoembryonic antigen)	Hu	B1.1/CD66	Mouse IgG_{2a},κ	FCM	PE	100 tests	**551480**	$260
CD108	Hu	KS-2	Mouse IgM, κ	FCM	Purified	0.1 mg	**552423**	$120
CD134 (OX40)	Hu	ACT35	Mouse IgG_1, κ	FCM	Cy-Chrome	100 tests	**551500**	$295
CD138	Hu	Mi15	Mouse IgG_1, κ	FCM	PE	100 tests	**552723**	$230
CD160	Hu	BY55	Mouse IgM, κ	FCM	Purified	0.1 mg	**551887**	$120
CD172a/b (SIRPα/β)	Hu	SE5A5	Mouse IgG_1, κ	FCM	PE	100 tests	**552722**	$260
CD172b (SIRPβ)	Hu	B4B6	Mouse IgG_1, κ	FCM	PE	100 tests	**552602**	$260
CD180	Hu	G28-8	Mouse IgG_1, κ	FCM	Purified	0.1 mg	**551890**	$120
CD222	Hu	MEM-238	Mouse IgG_1, κ	FCM	Purified	0.1 mg	**551982**	$120
CD229	Hu	HLy9.1.25	Mouse IgG_1	FCM	Purified	0.1 mg	**552751**	$120
CD231 (SN1)	Hu	H1-A12	Mouse IgG_1	FCM	FITC	100 tests	**551897**	$230
TAP2	Hu	TAP2.17	Mouse IgG_1, κ	FCM	FITC Set	100 tests	**551293**	$310

Mouse Cell Surface Molecules

DESCRIPTION	REACT	CLONE	ISOTYPE	APPS	FORMAT	SIZE	CAT. NO.	PRICE
CD3e (CD3 ε chain)	Ms	145-2C11	Armenian Hamster IgG1, κ	FCM	PE-Cy7	0.1 mg	**552774**	$325
CD4 (L3T4)	Ms	RM4-5	Rat (DA) IgG_{2a},κ	FCM	PE-Cy7	0.1 mg	**552775**	$325
CD11b	Ms	M1/70	IgG_{2b}, κ	FCM	PE-Cy7	0.1 mg	**552850**	$325
CD19	Ms	1D3	IgG_{2a}, κ	FCM	PE-Cy7	0.1 mg	**552854**	$325
CD36	Ms	CRF D-2712	Mouse (*CD36*$^{-1}$) IgA, κ	FCM, IF, IP	Purified	0.5 mg	**552544**	$150
CD45R/B220	Hu, Ms	RA3-6B2	Rat IgG_{2a}, κ	FCM	PerCP-Cy5.5	0.1 mg	**552771**	$325
CD45R/B220	Hu, Ms	RA3-6B2	Rat IgG_{2a}, κ	FCM	PE-Cy7	0.1 mg	**552772**	$325
CD157 (BP-3 Alloantigen)	Ms	BP-3	Mouse (*Mus spretus*) IgG_{2b}, κ	FCM	PE	0.1 mg	**552814**	$275
Dendritic Cells	Ms	33D1	Rat (SD) IgG_{2b}, κ	FCM	Biotin	0.5 mg	**552776**	$285
Notch1	Ms	mN1A	Mouse IgG_1, κ	IC/FCM	PE	0.1 mg	**552768**	$205

Product Supplement (continued from page 35)

Non-Human Primate

DESCRIPTION	REACT	CLONE	ISOTYPE	APPS	FORMAT	SIZE	CAT. NO.	PRICE
CCR6	Rh, Cyno	11A9	Mouse IgG_1, κ	FCM	PE	50 tests	**551773**	$140
HLA-DR	Bab, Rh, Cyno	G46-6	Mouse IgG_{2a}, κ	FCM	PerCP-Cy5.5	50 tests	**552764**	$315

Other Non-human Species

DESCRIPTION	REACT	CLONE	ISOTYPE	APPS	FORMAT	SIZE	CAT. NO.	PRICE
CD8b	Pig	295/33-25	Mouse (BALB/c) IgG_{2a}, κ	Cyt, FCM, IHC (Fr)	Purified	0.1 mg	**552769**	$150

Rat Cell Surface Molecules

DESCRIPTION	REACT	CLONE	ISOTYPE	APPS	FORMAT	SIZE	CAT. NO.	PRICE
RT6.1	Rat	P4/16	Rat (AUG a-PVG) IgG_{2b}, κ	FCM	Purified	0.1 mg	**552725**	$95

Isotype Controls

DESCRIPTION	CLONE	ISOTYPE	APPS	FORMAT	SIZE	CAT. NO.	PRICE
Hamster IgG3, κ (anti-KLH)	E36-239	Armenian Hamster IgG3, κ	FCM	PE	0.1 mg	**552144**	$165
Hamster IgG_1,κ (anti-TNP)	A19-3	Armenian Hamster IgG1,κ	FCM	PE-Cy7	0.1 mg	**552811**	$205
Mouse IgG_{2a}, κ (Specific for TNP)	G155-178	Mouse IgG_{2a}, κ	FCM	PerCP-Cy5.5	50 tests	**552577**	$150
Rat IgG_{2a}, κ	R35-95	Rat (LOU) IgG_{2a}, κ	FCM	APC-Cy7	0.1 mg	**552770**	$205
Rat IgG_{2a}, κ	R35-95	Rat (LOU) IgG_{2a}, κ	FCM	PE-Cy7	0.1 mg	**552784**	$205
Rat IgG_{2b}, κ	A95-1	Rat (LOU) IgG_{2a}, κ	FCM	PE-Cy7	0.1 mg	**552849**	$205
Rat IgG_{2b}, κ	A95-1	Rat (LOU) IgG_{2b}, κ	FCM	APC-Cy7	0.1 mg	**552773**	$205

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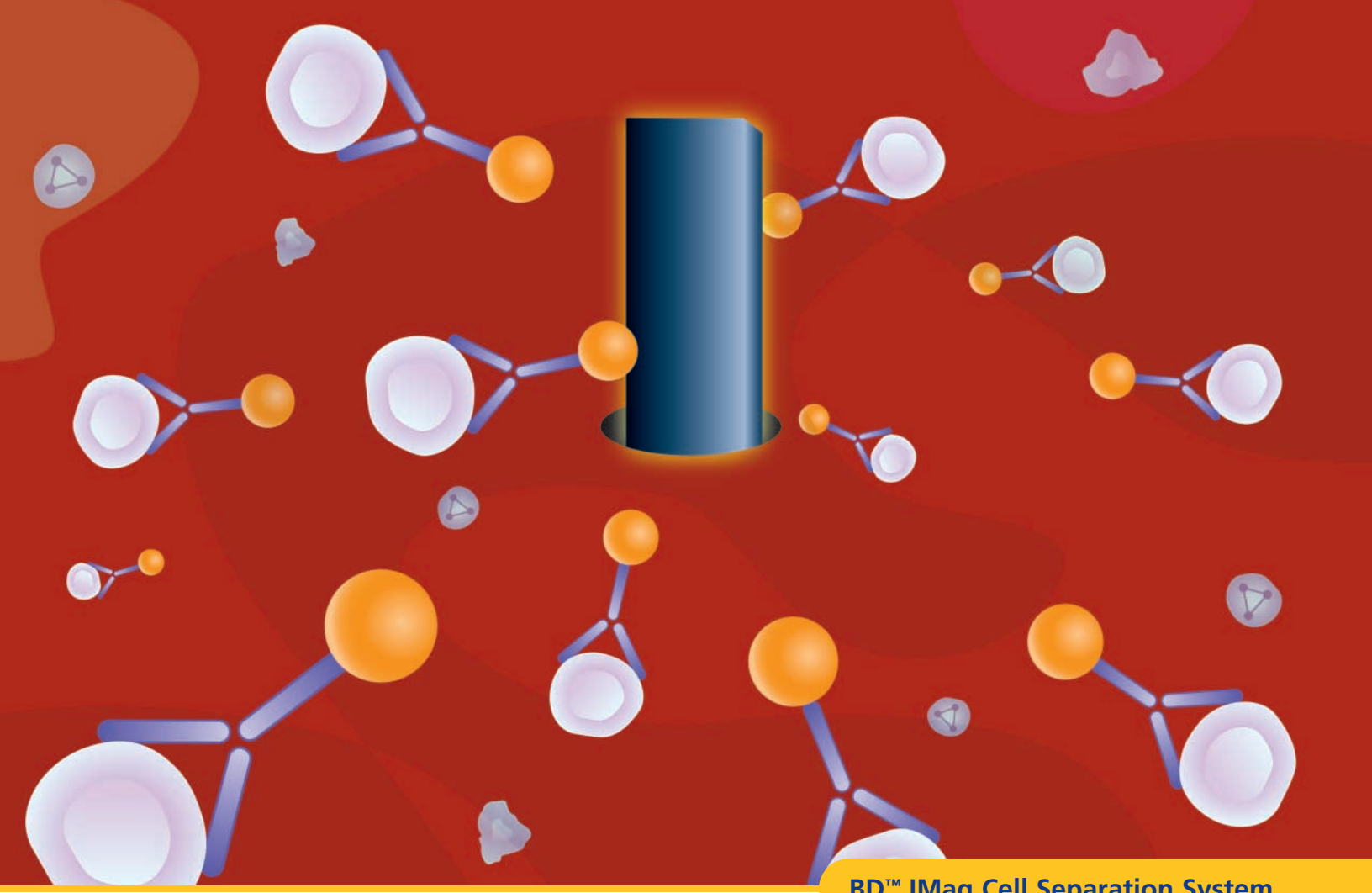

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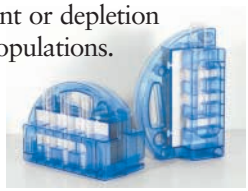

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- Complete product listing
- Technical resources
- Alphabetical and numeric indices

Where We'll Be in Fall 2002

When	What	Where
October 4 - 6	Great Lakes International Imaging & Flow Cytometry Associaton (GLIIFCA)	Detroit, MI
October 6 - 11	Joint International Cytokine Society (ICS) and Society for Leukocyte Biology (SLB) Meeting	Torino, ITALY
October 11 - 13	Bi-Annual Clinical Flow Cytometry Course	Keystone, CO
October 13 - 16	Clinical Applications of Cytometry (CAC)	Keystone, CO
October 17 - 18	National Institutes of Health (NIH) Research Festival	Bethesda, MD
October 19 - 23	American Society for Histocompatibility and Immunogenetics (ASHI)	Nashville, TN
October 26 - 29	American Association of Blood Banks (AABB)	Orlando, FL
October 27 - 31	Eleventh North American International Society for the Study of Xenobiotics Meeting (ISSX)	Orlando, FL
November 2 - 7	Society for Neuroscience (SFN)	Orlando, FL
November 10 - 14	American Association of Pharmaceutical Scientists (AAPS)	Toronto, CANADA
November 22 - 24	Human Proteom Organization (HUPO)	Paris, FRANCE
November 22 - 25	Midwest Autumn Immunology Conference (AIC)	Chicago, IL
December 6 - 10	American Society of Hematology (ASH)	Philadelphia, PA
December 14 - 18	American Society for Cell Biology (ASCB)	San Francisco, CA

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