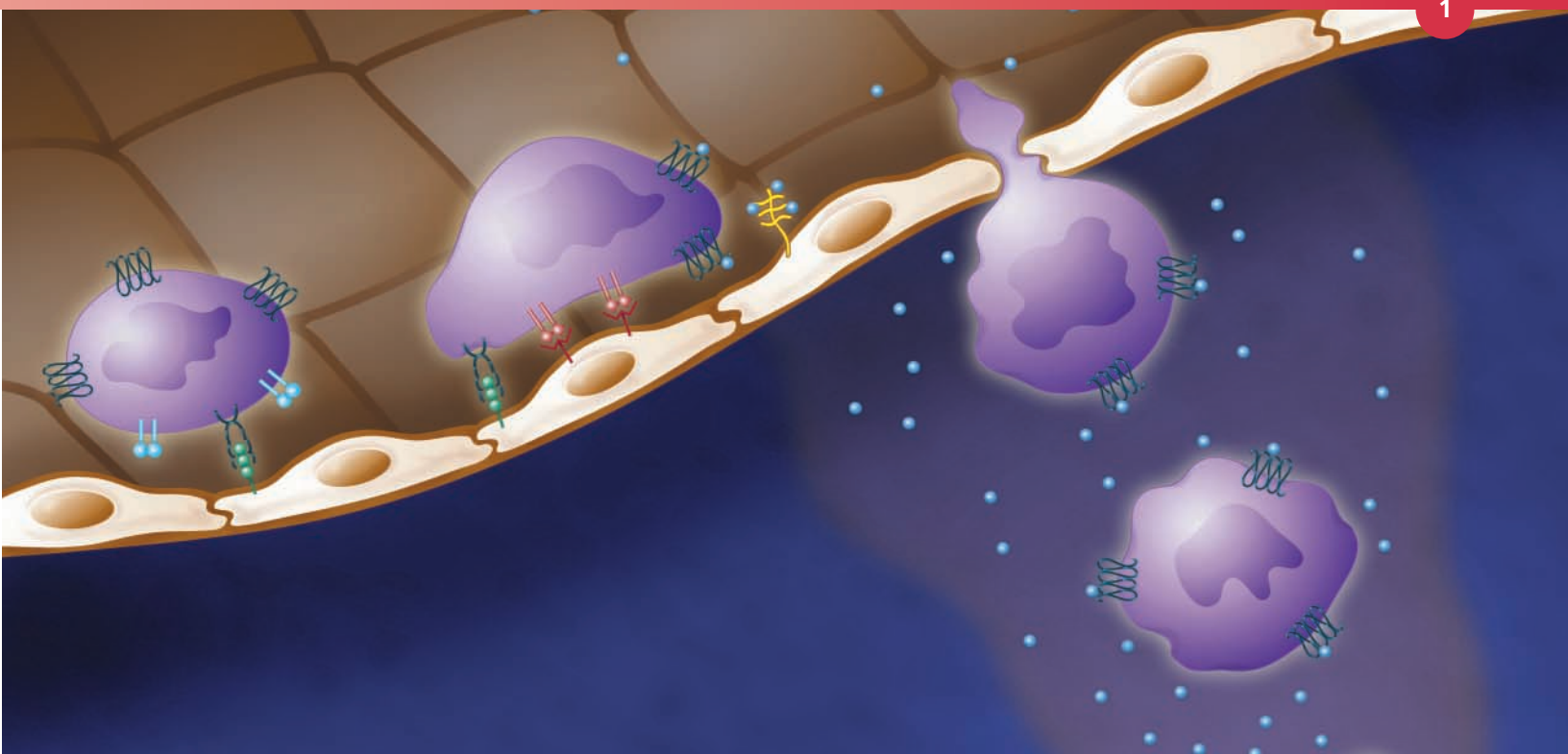


HotLines

VOL 6 NO 2 FALL 2001

1



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CCR7 and Lymphocyte Trafficking

By Jing Ping Shih, Ph.D.

Chemokines are a group of small (8 to 14 kDa), structurally related, mostly basic and heparin-binding cytokines. Over 45 chemokines have been identified in humans. Based on the arrangement of the first two amino-terminal cysteine residues, chemokines can be subdivided into four families: CC (CCL1 - CCL28), CXC (CXCL1 - CXCL16), C (XCL1) and CX3C (CX3CL1)¹. All chemokines exert their biological function via a group of

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Continued on page 2

CCR7 and Lymphocyte Trafficking *(continued from cover)*

seven-transmembrane, G protein-coupled receptors (GPCRs). Like chemokines, their receptors can also be divided into 4 families based on the ligands they bind to: CC chemokine receptors (CCR1 – CCR11), CXC chemokine receptors (CXCR1 – CXCR6), C chemokine receptor (XCR1) and CX3C chemokine receptor (CX3CR1)².

Chemokines were originally thought to attract granulocytes and monocytes and to be involved in acute and chronic inflammatory responses³. Recently, newly emerging chemokines have been shown to be involved in controlling leukocyte trafficking. These new chemokines are functionally and genetically distinct from the classical “inflammatory chemokines” and may be classified as “lymphoid chemokines” or “homeostatic chemokines”⁴.

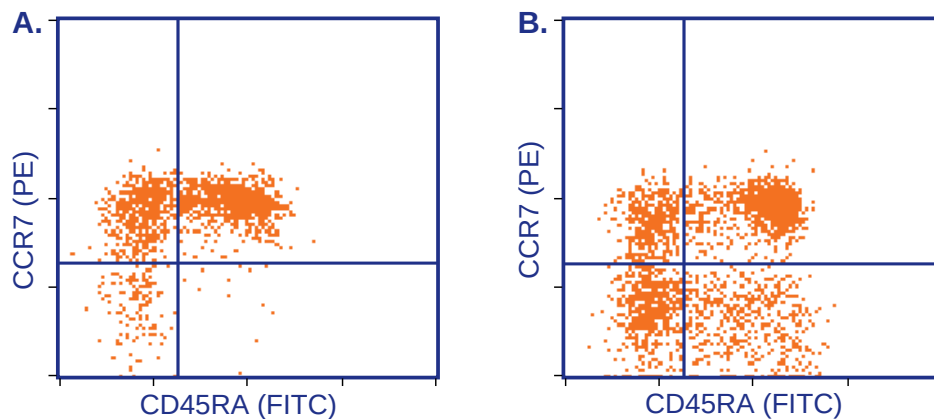
The interaction of CCR7 and its ligands SLC (CCL21) and ELC (CCL19) demonstrates the critical role of the chemokine system in the transmigration of peripheral T lymphocytes to secondary lymphoid tissues^{5,6}. When the circulating naïve T cells enter the lymph nodes, they have to make contact with high endothelial venules (HEV) via CD62L-PNAd interaction. This allows the CCR7 to interact with its ligand SLC which is constitutively produced by HEV. The binding of SLC to CCR7 activates the integrin system that induces the cell adhesion and transmigration process into the lymph nodes⁷. The T cells then migrate toward the T zones via gradients of another CCR7 ligand, ELC, which is expressed within the T zones. Dendritic cells enter the secondary lymphoid tissues through the afferent lymphatics and migrate toward the T zones via a gradient of SLC and ELC⁸.

The critical roles of CCR7 and its ligand in these processes were further demonstrated in CCR7-deficient mice⁹ and plt mice¹⁰⁻¹², respectively. In CCR7-deficient mice, there were significantly reduced number of T cells present in the secondary lymphoid tissues and the structure of their lymph nodes was disorganized⁹. These mice also revealed an impairment in T cell-dependent immune responses such as delayed-type hypersensitivity and antibody production⁹. The plt mice are deficient in SLC expression in HEV^{11,12} and have phenotypes similar to CCR7-deficient mice. By bringing together the T cells, B cells and dendritic cells within secondary lymphoid

tissues, the CCR7-SLC/ELC interaction illustrates the vital role of the chemokine system in leukocyte trafficking and immune responses.

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Detection of CCR7 expression on CD4⁺ and CD8⁺ human PBL by purified anti-human CCR7 antibody 2H4. Human PBMC were stained with 4.0 µg of purified 2H4 followed by biotinylated anti-mouse IgM (Cat. No. 02082D), streptavidin-PE (Cat No. 13025D) and anti-human CD45RA-FITC (Cat. No. 31264X). The data shown are derived from the CD4⁺ (based on staining with anti-human CD4-APC, Cat. No. 30159X, **Panel A**) and CD8⁺ (based on staining with anti-human CD8-APC, Cat. No. 30329X, **Panel B**) lymphocyte-gated populations displayed as bivariate dot plots.

CCR7 Reagents and Other New Chemokine Receptor Antibodies

New Cat. No.	Cat. No.	Product Specificity	Clone	Format	Size	
550937	24431C	Human CCR7	2H4	Purified	0.25 mg	
551852	25561A	Mouse CXCR4	2B11/CXCR4	Purified	0.1 mg	NEW
551854	25562X	Mouse CXCR4	2B11/CXCR4	Biotin	100 tests	NEW
551856	25564X	Mouse CXCR4	2B11/CXCR4	FITC	100 tests	NEW
551855	25565X	Mouse CXCR4	2B11/CXCR4	PE	100 tests	NEW
551413	25321A	Human CXCR4 (Fusin)	1D9	Purified	0.1 mg	NEW
551504	25322D	Human CXCR4 (Fusin)	1D9	Biotin	0.5 mg	NEW
551510	25325B	Human CXCR4 (Fusin)	1D9	PE	0.2 mg	NEW
551121	25141A	Human CCR4	1G1.1	Purified	0.1 mg	
551266	25142D	Human CCR4	1G1	Biotin	0.5 mg	
551120	25145B	Human CCR4	1G1.1	PE	0.2 mg	
559560	23531D	Human CCR6	11A9	Purified	0.5 mg	
559561	23532D	Human CCR6	11A9	Biotin	0.5 mg	
559562	23535B	Human CCR6	11A9	PE	0.2 mg	
551773	71975L	Human CCR6	11A9	PE	50 test	

NEW from the Cytometric Bead Array Program at BD Biosciences Pharmingen

By Dara Grantham Wright and Jerry Wilson

The BD Cytometric Bead Array (CBA) combines a series of particles of discrete fluorescence intensity with matched antibody pairs to allow for simultaneous detection of multiple soluble analytes on a flow cytometer. The flow cytometer's capacity to discriminate particles on the basis of size and color enables CBA assays to yield truly multiplexed results from a single sample (Figure 1). The combination of the BD CBA Kits, a flow cytometer, and the BD CBA Software provide the researcher with a complete multiplex analysis system for their research needs.

The BD CBA System is continually growing and improving. Recently, the Human Th1/Th2 Cytokine CBA Kit was modified to incorporate a new capture antibody for human IFN- γ , which has significantly improved the signal and sensitivity for the human IFN- γ assay. This is just the beginning of the improvements and expansion to the CBA portfolio of products.

Did you know that the Human CBA Kits also detect some non-human primate cytokines?

Many CBA customers have determined that they are able to detect positive signals for rhesus and cynomolgus macaque samples when using the Human Th1/Th2 Cytokine CBA Kit. CBA results have been confirmed by ELISA using the CBA antibody pairs and activated cell culture samples from both rhesus and cynomolgus macaques. The cross-reactivity of Human CBA assays with non-human primate (NHP) analytes have not yet been normalized to native NHP proteins, so direct quantitation is not currently available (Table 1).

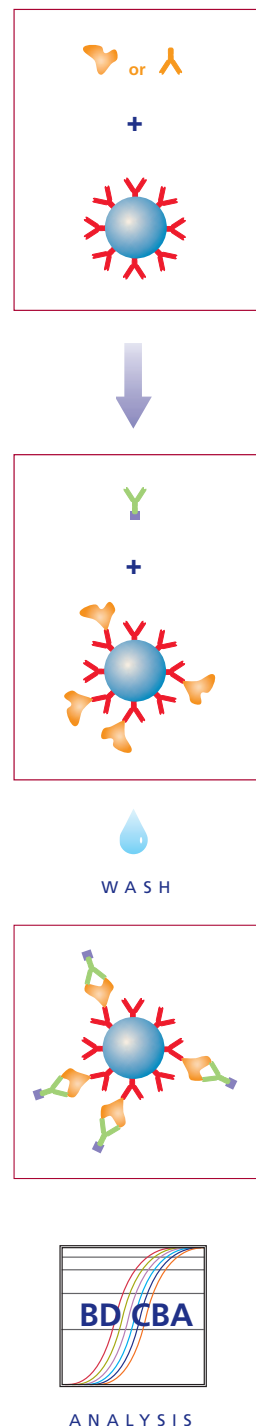


Figure 1

Table 1. BD CBA cross-reactivity with non-human primate analytes.

Cat. No.	Description	Rhesus and Cynomolgus Cross-reactivity
550749	Human Th1/Th2 Cytokine CBA Kit	Interleukin (IL)-4, IL-5, TNF- α , IFN- γ
551809	Human Th1/Th2 Cytokine CBA Kit – II	Interleukin (IL)-4, IL-6, TNF- α , IFN- γ
551811	Human Inflammation CBA Kit – I	Interleukin (IL)-8, IL-6, TNF- α (IL-1 β and IL-12p70 not yet tested)

Table 2. BD CBA Software compatibility

	Version 1.0	Version 1.1	Version 1.2
Excel 5	x	x	
Excel 98	x	x	
Excel 2000 (PC only)			x
Excel 2001		x	
OS 8.1 to OS 9	x	x	
Windows 98			x
Windows NT 4.10			x
Mac PowerPC $\geq$ 7000	x	x	
Macintosh G3/G4	x	x	
PC			x
Int'l Mac OS versions		x	

Instrument setup is even easier on a dual-laser BD FACSCalibur™

While the fluorescently labeled particles in the BD CBA assays are designed to be excited by the 488 nm laser common to all BD flow cytometers, they can also be excited by the red diode laser on dual-laser BD FACSCalibur instruments. Use of the red diode laser for exciting the CBA particles and detection of particle emission on the FL4 channel simplifies the instrument set up procedure and reduces the need for fluorescence compensation. An instrument set up protocol and template for dual-laser FACSCalibur instruments, along with many other updated files for CBA, can be found on CBA page at the following URL:

<http://www.bdbiosciences.com/pharming/cba/>

New versions of the BD CBA Software add compatibility with all current versions of MS Excel®

The BD CBA Software has been upgraded to version 1.1 and version 1.2 to add increased compatibility with multiple operating systems and MS Excel versions (Table 2).

NEW from the Cytometric Bead Array Program at BD Biosciences Pharmingen

(continued from page 5)

New BD CBA Assay Kits and Products

Mouse Th1/Th2 Cytokine CBA Kit

50 tests Cat. No. 551287

Simultaneous detection of Mouse IL-2, IL-4, IL-5, IFN- γ , and TNF- α from serum or supernatant samples. Complete kit containing sufficient reagents to run 50 samples.

Now Available

Human Th1/Th2 Cytokine CBA Kit – II (Now with IL-6)

50 tests Cat. No. 551809

Simultaneous detection of Human IL-2, IL-4, IL-6, IL-10, TNF- α , and IFN- γ from serum, plasma, or supernatant samples. Complete kit containing sufficient reagents to run 50 samples.

Now Available

Human Inflammatory Cytokine CBA Kit – I

50 tests Cat. No. 551811

Simultaneous detection of Human IL-8, IL-1 β , IL-6, IL-10, TNF- α , and IL-12p70 from serum, plasma, or supernatant samples. Complete kit containing sufficient reagents to run 50 samples.

Available October 2001

Human Th1/Th2 Cytokine Standards

1 vial Cat. No. 2428KC

A single-use vial containing lyophilized recombinant human IL-2, IL-4, IL-5, IL-6, IL-10, TNF- α , and IFN- γ for use as a standard in the Human Th1/Th2 Cytokine CBA Kits (Cat. Nos. 550749 and 551809).

Available October 2001

Human Th1/Th2 Cytokine CBA

50 tests Cat. No. 550749

Simultaneous detection IL-2, IL-4, IL-5, IL-10, TNF- α , and IFN- γ from serum, plasma, or supernatant samples. Complete kit containing sufficient reagents to run 50 samples.

Now Available

Mouse Ig Isotyping CBA

100 tests Cat. No. 550026

Simultaneous immunoglobulin isotype profile, including light chain analysis, of mouse IgG₁, IgG_{2a}, IgG_{2b}, IgG₃, IgA, IgM, and IgE.

Now Available

BD CBA Software

1 CD (v 1.1 and 1.2) Cat. No. 550065

The BD CBA Software enables rapid analysis of CBA data files for generation of standard curves and sample value reporting.

Now Available

Did you know you could increase throughput and decrease hands-on time by combining the BD CBA with the New BD Multiwell™ AutoSampler System?

Add walk-away automation to your CBA assays with the new Multiwell™ AutoSampler for use with BD Biosciences flow cytometers.

- Provides walk-away sample introduction from a variety of multiwell plates
- Includes Multiwell Plate Manager software for acquisition and data analysis
- Equipped with the FACSFlow™ Supply System for hours of hands-free operation
- Compatible with instruments with the FACSTM Loader option
- Installs easily beneath the cytometer with no additional bench space required

Features:

- Flexible acquisition from 96- or 384-well plates, both standard and deep-well
- User-definable sample volume, mixing, and washing for optimal performance
- Innovative Cytometer Interface Unit (CIU) provides consistent sample throughput
- New graphical user interface for test setup, acquisition control, and data retrieval
- Colorful analysis software shows results at a glance

Specifications:

- Mixing: selectable for 0–3 mix repetitions, definable from 10–250 mL
- Washing: selectable for 0–3 wash repetitions
- Sample volume: definable from 25–250 mL
- Sample carry over: < 1% with one wash cycle between cell samples
- Bench space: no additional bench space required; FACScan™ cytometers require lift kits (included).



Visit the CBA homepage for the most current CBA system updates:

<http://www.bdbiosciences.com/pharmingen/cba/>

The ImmunoSpot™ Series 1 Analyzer

By Alexey Karulin, Ph.D., Tameem Ansari, M.Sc,
David Sehy, M.Sc, and Paul Lehmann, M.D., Ph.D.

The ImmunoSpot™ Analyzer was developed specifically for performing complex ELISPOT analyses, and has matured over a five-year testing period in a leading immunology research laboratory. The ImmunoSpot™ Analyzer has been validated in more than seventeen publications and has been selected by the NIH as the reference tool for ELISPOT image analysis. The stand alone system is equipped with a camera, a lens, a light source, a high precision X-Y stage, a PC with monitor and keyboard, and the ImmunoSpot™ application program. Satellite systems are available for the analysis of raw data images.

The ImmunoSpot™ Analyzer has been optimized to meet the needs of researchers who demand sophistication and flexibility from a highly efficient, user-friendly system for the acquisition and analysis of ELISPOT data.



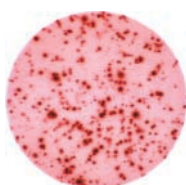
Figure 1. The ImmunoSpot™ Series 1 Analyzer (CTL Analyzers LLC, Cleveland, OH)



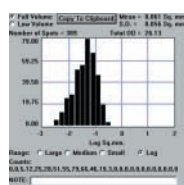
ImmunoSpot™ ELISPOT plates and ImmunoSpot™ Series I Analyzer are trademarks of Cellular Technology Limited, Cleveland, Ohio.

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

For more information, please visit www.immunospot.com



Panel A



Panel B

Figure 2. ELISPOT analysis of human IL-10-secreting cells. Primed human PBMCs were restimulated in culture with PMA and ionomycin in the microwell of an ImmunoSpot™ ELISPOT plate, which was precoated with unlabelled NA/LE™ anti-human IL-10 antibody (Human IL-10 ELISPOT Reagent Set, Cat. No. 551018). Biotinylated anti-human IL-10 antibody was used to detect the captured IL-10. Spots were visualized using avidin-HRP enzyme and AEC substrate, followed by image analysis and spot enumeration using an ImmunoSpot™ Series 1 Analyzer (CTL Analyzers LLC, Cleveland, OH). The scanned image is shown in **Panel A**. The spot size distribution of the PMA and ionomycin-induced response, as measured by image analysis, is shown in **Panel B**.

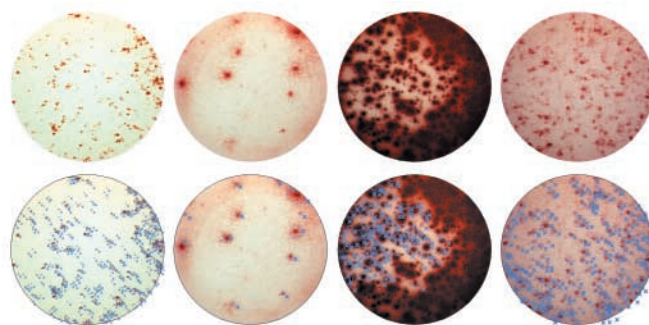


Figure 3. Sophisticated software for dealing with complex and difficult assay results. The ImmunoSpot™ analysis software has solutions for the many different problems that arise in the course of performing ELISPOT assays. From left to right, it can handle: tiny, pointlike spots that still need to be separated; fuzzy spots on irregular backgrounds; estimation of spots in overdeveloped areas based on readable portions (not by area normalization); and spattery spots on a grainy background.

ELISPOT Instrumentation and Software

For further information about the ImmunoSpot™ Series 1 Analyzer or Satellite and to arrange for a demonstration, please contact your local BD Biosciences reagent sales consultant.

ImmunoSpot™ Series 1 Analyzer

for scanning of plates and fully automated high resolution ELISPOT image analysis

- Acquisition Unit
- PC System with Windows
- 4x/16/CD-R, frame grabber
- 17" Monitor
- ImmunoSpot™ Software
- Uninterruptible Power Supply
- User's Manual

ImmunoSpot™ Series 1 Satellite

for fully automated high resolution ELISPOT image analysis

- PC System with Windows
- 17" Monitor
- ImmunoSpot™ Software
- User's Manual

ImmunoSpot™ software

Unique features of the ImmunoSpot™ Series 1 Analyzer

Fast, fully automated acquisition and storage of high-resolution images

The ImmunoSpot™ Analyzer captures images of all standard ELISPOT 96-well microtiter plates in fully automated mode, and saves those images directly to CD. The images may be sent to an ImmunoSpot™ Satellite when the acquisition unit of the system is busy capturing images, to permit a high throughput of data analysis. The Analyzer provides imaging capability to single-cell resolution.

Fully automatic well centering

The ImmunoSpot™ Analyzer centers each well under the camera to accommodate variation from plate to plate and automatically adjust the light intensity ensuring that the image of each and every well is captured in its entirety.

Software functions for coping with the complexities of everyday use

The ImmunoSpot™ Analyzer offers a range of user-definable parameters for dealing with real world analysis of plates: Background Balance, Overdeveloped Area Processing, Sensitivity Adjustment, Spot Size, Spot Separation Tolerance, Detail, and Diffuse Spot Counting — all adjustable to help the researcher customize analysis.

Custom imaging

The ImmunoSpot™ Analyzer permits compilation of stored images in a variety of ways. During automatic image acquisition of images the Analyzer stores the image of each well in its own file and then makes a composite image of the whole plate. The storage of well images in discrete files enables the researcher to retrieve any number of files and manipulate them into a custom array.

Automated analysis of data

Once the raw images are acquired and the parameters are set, the analysis of images is fully automatic, yielding both a spot count and a histogram. Working in virtual mode, one may re-analyze raw images according to different parameters, again automatically. The accuracy of automatic analysis has been validated to 1%, demonstrating that objectivity and reproducibility are assured. Data and analyses are exportable to spreadsheets for generating figures and tables.

Versatility

The ImmunoSpot™ Analyzer may be used alone or networked with any number of Satellites. Furthermore, the ImmunoSpot™ Analyzer may be coupled with a robotic plate loader/stacker for high volume applications.

NEW Interferon- γ ELISPOT Assays

By Qi Guan, David Sehý, M.S. and David Ernst, Ph.D.

The enzyme-linked immunospot (ELISPOT) assay is a powerful tool for detecting and analyzing individual cells that secrete a particular protein *in vitro*¹. Although originally developed for analyzing specific antibody-secreting cells^{2,3}, the assay has been adapted for measuring the frequencies of cells that produce and secrete a variety of other effector molecules such as cytokines^{4,5,6}. The ELISPOT assay has the advantage of being capable of detecting cytokine-producing cells from both activated naïve and memory T cell populations, as well as a variety of other cell types. Cytokine release can be detected at the single cell level, allowing direct determination of cytokine-producing cell frequencies⁵. Furthermore, this assay has been found to be more sensitive than ELISA and intracellular staining^{1,7,8}. The sensitivity of the assay enables measurement of even very low frequencies of cytokine-producing cells (*e.g.*, 1/300,000)¹. Recent developments in assay plate design and in ELISPOT plate-reader instrumentation have significantly improved the utility of the ELISPOT method for objective and rapid analysis of cytokine-producing cells¹.

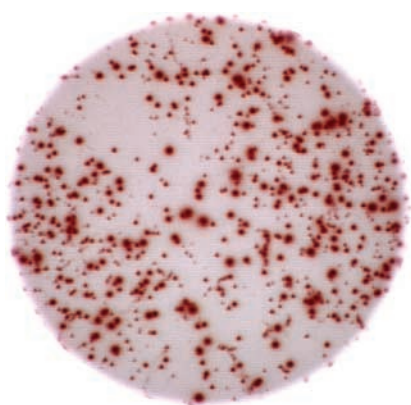
The ELISPOT derives its specificity and sensitivity by employing high affinity capture and detection antibodies and enzyme-amplification. Since 1989, Pharmingen has produced and supplied high quality antibody pairs for cytokine analysis. Numerous publications cite BD Pharmingen™ cytokine ELISA pairs for ELISPOT assay, including *Current Protocols in Immunology* (John Wiley & Sons, Inc., NY). Recent collaborations with CTL Analyzers LLC (www.immunospot.com; Cleveland, OH), the developers of state-of-the-art ImmunoSpot™ plate reader instrumentation and software, have led to the identification of novel, optimal antibody clone pairings for ELISPOT assays. In the course of screening many antibody clones for the ELISPOT application, we ascertained that some antibody pairs which perform very well for ELISA are not necessarily effective for ELISPOT assay. ELISPOT-compatible antibody clones were selected based on sensitivity and ability to produce discrete and densely-colored spots. In some cases, best ELISPOTs were found

to result from cocktails made from multiple capture antibody clones combined. To avoid the effects of sodium azide and endotoxin on cultured cells, the BD Pharmingen™ ELISPOT capture antibodies are special formulations which contain no sodium azide and minimal endotoxin (NA/LE™).

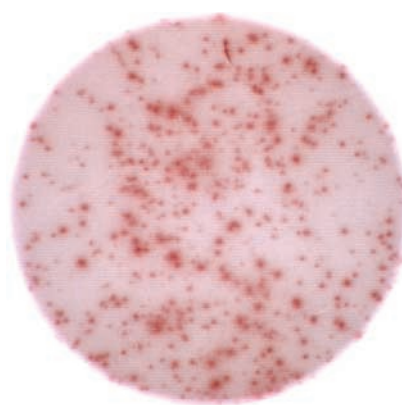
The IFN- γ ELISPOT assay is one important tool for characterization of T cell effector function. To identify the best IFN- γ ELISPOT reagents, we have screened a panel of new anti-mouse and anti-human IFN- γ antibody clones in ELISPOT assays with a variety of target cell preparations, including naïve T cells, memory T cells, and cell lines. We are proud to announce that BD Biosciences Pharmingen now has available specially screened and formulated high sensitivity antibody pairs for human IFN- γ and mouse IFN- γ ELISPOT assay applications. These ELISPOT-compatible antibody pairs have also proven to be very effective for conventional sandwich ELISA.

The IFN- γ ELISPOT reagents are to be available in three different formats, which provide varying degrees of flexibility and time saving convenience: Pairs, Sets, and Kits.

- **ELISPOT Reagent Pairs:** Sufficient antibody for 5 plates of assays. These reagents are tested with, and recommended for use with, high sensitivity/low background ImmunoSpot™ ELISPOT plates available from CTL Analyzers LLC (Cleveland, OH). www.immunospot.com.
- **ELISPOT Reagent Sets:** 10 ImmunoSpot™ ELISPOT plates (not pre-coated with antibody), along with sufficient antibody for 10 plates of assays.
- **ELISPOT Kits:** Designed for maximal time savings and convenience, supplying 2 plates, precoated with antibody, along with all buffers, substrates, enzymes, and detection reagents needed for those 2 plates of assays.



Panel A



Panel B

Figure 1. Superior Human IFN- γ ELISPOTS Are Obtained Using a novel anti-human IFN- γ Antibody for Capture. Primed human PBMC were restimulated (overnight) with PMA (5 ng/ml; Sigma, Cat. No. P-8139) and ionomycin (500 ng/ml; Sigma, Cat. No. I-0634) in the microwell of an ImmunoSpot™ ELISPOT plate that was pre-coated with the NA/LE anti-human IFN- γ . Biotinylated anti-human IFN- γ antibody was used to detect the captured IFN- γ . Spots were visualized using avidin-HRP enzyme and AEC substrate. Image analysis and spot enumeration were carried out using the ImmunoSpot™ Series I Analyzer (CTL Analyzers LLC, Cleveland, OH). Panels A and B were derived from experiments conducted using the same activated cells in the same ImmunoSpot™ plate.

Panel A: Human IFN- γ ELISPOT Set (BD Biosciences Pharmingen, Cat. No. 2554KI).

Panel B: Alternative human IFN- γ ELISPOT reagent pairing.

BD Pharmingen™ ELISPOT Products

ELISPOT Reagent Pairs

- Unlabelled anti-cytokine capture antibody (NA/LE™ format); sufficient reagent for coating 5 plates
- Biotinylated anti-cytokine detection antibody; sufficient reagent for 5 plates
- Certificate of Analysis, providing lot-specific optimal reagent concentrations

ELISPOT Reagent Sets

- 10 ImmunoSpot™ ELISPOT plates
- Unlabelled anti-cytokine capture antibody (NA/LE™ format); sufficient reagent for coating 10 plates
- Biotinylated anti-cytokine detection antibody; sufficient reagent for 10 plates

- Avidin horseradish peroxidase; sufficient for 10 plates
- Certificate of Analysis, providing lot-specific optimal reagent concentrations

ELISPOT Kits

- 2 pre-coated ImmunoSpot™ ELISPOT plates
- Biotinylated anti-cytokine detection antibody; sufficient reagent for 2 plates
- Avidin horseradish peroxidase; sufficient for 2 plates
- Certificate of Analysis, providing lot-specific optimal reagent concentrations
- Assay diluent
- Wash Buffer
- AEC substrate

NEW Interferon- γ ELISPOT Assays *(continued from page 11)*

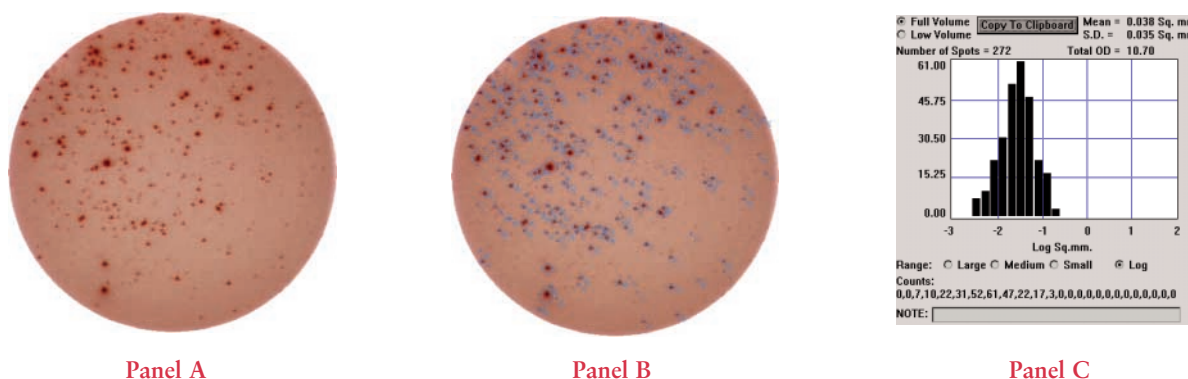


Figure 2. Mouse IFN- γ ELISPOT Analysis Using a novel anti-mouse IFN- γ Antibody for Capture. BALB/c mouse spleen cells were stimulated with PMA (5 ng/ml; Sigma, Cat. No. P-8139) and ionomycin (500 ng/ml; Sigma, Cat. No. I-0634), and incubated in an ImmunoSpot™ ELISPOT plate pre-coated with anti-mouse IFN- γ mAb (BD Biosciences Pharmingen; Mouse IFN- γ ELISPOT Set, Cat. No. 2501KI) overnight. The plate was washed, biotinylated detection mAb was added and incubated for 2 hours. After washing the plate microwells, Avidin-HRP was added and incubated for 1 hour. (1:100, BD Biosciences Pharmingen) The plate was completely washed again and developed with AEC substrate (3-amino-9-ethyl carbazole, Sigma, Cat. No. A-5754). Image analysis and spot enumeration were carried out using the ImmunoSpot™ Series I Analyzer (CTL Analyzers LLC, Cleveland, OH).

Panel A: Microwell image of mouse IFN- γ spots.

Panel B: Microwell image of mouse IFN- γ spots detected and enumerated by ImmunoSpot™ software (CTL Analyzers LLC, Cleveland, OH).

Panel C: The spot size distribution of the PMA and ionomycin-induced response, as measured by image analysis.

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ELISPOT Reagent Product List

ELISPOT Reagent Sets

New Cat. No.	Cat. No.	Description	Format	Size
551083	2510KI	Mouse IFN- γ	Set	Antibody reagents, Av-HRP, and 10 uncoated ImmunoSpot™ plates
551849	2554KI	Human IFN- γ	Set	Antibody reagents, Av-HRP, and 10 uncoated ImmunoSpot™ plates
551017	2446KI	Mouse IL-4	Set	Antibody reagents, Av-HRP, and 10 uncoated ImmunoSpot™ plates
551084	2511KI	Human IL-4	Set	Antibody reagents, Av-HRP, and 10 uncoated ImmunoSpot™ plates
551075	2450KI	Mouse IL-5	Set	Antibody reagents, Av-HRP, and 10 uncoated ImmunoSpot™ plates
551085	2512KI	Human IL-5	Set	Antibody reagents, Av-HRP, and 10 uncoated ImmunoSpot™ plates
551491	2538KI	Mouse TNF- α	Set	Antibody reagents, Av-HRP, and 10 uncoated ImmunoSpot™ plates
551446	2534KI	Human TNF- α	Set	Antibody reagents, Av-HRP, and 10 uncoated ImmunoSpot™ plates
551076	2509KI	Mouse IL-2	Set	Antibody reagents, Av-HRP, and 10 uncoated ImmunoSpot™ plates
551282	2515KI	Human IL-2	Set	Antibody reagents, Av-HRP, and 10 uncoated ImmunoSpot™ plates
551445	2533KI	Mouse IL-10	Set	Antibody reagents, Av-HRP, and 10 uncoated ImmunoSpot™ plates
551018	2449KI	Human IL-10	Set	Antibody reagents, Av-HRP, and 10 uncoated ImmunoSpot™ plates

ELISPOT Reagent Pairs

New Cat. No.	Description	Format	Size
551881	Mouse IFN- γ	Pairs	Antibody reagent for 5 plates of assays
551873	Human IFN- γ	Pairs	Antibody reagent for 5 plates of assays
551945	Monkey IFN- γ	Pairs	Antibody reagent for 5 plates of assays
551878	Mouse IL-4	Pairs	Antibody reagent for 5 plates of assays
551885	Human IL-4	Pairs	Antibody reagent for 5 plates of assays
551949	Monkey IL-4	Pairs	Antibody reagent for 5 plates of assays
551880	Mouse IL-5	Pairs	Antibody reagent for 5 plates of assays
551886	Human IL-5	Pairs	Antibody reagent for 5 plates of assays
551946	Monkey IL-5	Pairs	Antibody reagent for 5 plates of assays
551875	Mouse TNF- α	Pairs	Antibody reagent for 5 plates of assays
551882	Human TNF- α	Pairs	Antibody reagent for 5 plates of assays
551948	Monkey TNF- α	Pairs	Antibody reagent for 5 plates of assays
551876	Mouse IL-2	Pairs	Antibody reagent for 5 plates of assays
551884	Human IL-2	Pairs	Antibody reagent for 5 plates of assays
551947	Monkey IL-2	Pairs	Antibody reagent for 5 plates of assays
551874	Mouse IL-10	Pairs	Antibody reagent for 5 plates of assays
551883	Human IL-10	Pairs	Antibody reagent for 5 plates of assays

Complete ELISPOT Kits

New Cat. No.	Description	Format	Size
Please inquire	Human IFN- γ	Kit	2 pre-coated ImmunoSpot™ plate and all necessary reagents and buffers
Please inquire	Human IL-4	Kit	2 pre-coated ImmunoSpot™ plate and all necessary reagents and buffers
Please inquire	Human IL-5	Kit	2 pre-coated ImmunoSpot™ plate and all necessary reagents and buffers
Please inquire	Human TNF- α	Kit	2 pre-coated ImmunoSpot™ plate and all necessary reagents and buffers
Please inquire	Human IL-2	Kit	2 pre-coated ImmunoSpot™ plate and all necessary reagents and buffers

Ancillary Reagents

New Cat. No.	Description	Size
551951	AEC Substrate	100 ml, sufficient for 10 plates of assays
551950	Avidin-HRP	1 ml, sufficient for 10 plates of assays

Dendritic Cells and Antigen Processing and Presentation

By Enoc J. Hollemweguer, Ph.D., Bruce Koppelman, Ph.D., Li Li, Ph.D., Jay Dong, M.D., M.S.

One of the most important functions of the adaptive immune response is antigen recognition. Without the ability of cells to recognize antigens in a specific fashion, the immune system would be unable to distinguish self from non-self. Special cells are capable of processing extracellular pathogens and displaying them on their cell surface to be recognized by T cells. Infectious bacteria and other extracellular microorganisms are captured by macrophages, dendritic cells or B cells, internalized for further digestion, then presented as an MHC class II: peptide complex recognized by T-helper CD4⁺ cells. Intracellular parasites like viruses, on the other hand, can be presented by all nucleated cells and are processed through a different cellular compartment. These cells mostly present these pathogens as a MHC class I: peptide complex recognized by T-suppressor/cytotoxic CD8⁺ cells^{1,2}.

Dendritic Cells

Among the cells that capture and process antigens, dendritic cells have been described in the literature as being the most efficient². Dendritic cells (DC) are referred to as the professional antigen presenting cells (APC). Dendritic cells are a heterogeneous cell population that continuously derive from bone marrow stem cells. Following maturation, they reside in both lymphoid and non-lymphoid tissues. Liu *et al*³ described two human dendritic cell lineages. Pre-DC1 myeloid monocytes give rise to myeloid DC1 that produce a significant amount of IL-12 and thus induce Th1 responses in humans. Pre-DC2 plasmacytoid DC, which become lymphoid DC2, induce human Th2 responses. Both lymphoid and myeloid pre-DCs express toll-like receptors (TLR) and lectin molecules, but lymphoid DCs express TLR7 and TLR9 while myeloid DCs express only TLR2 and 4. Myeloid DC1s express large amount of mannose receptor, but DC2s do not⁴.

The development of bone marrow-derived DCs is highly dependent on their microenvironment. Several cytokines and growth factors have been identified as being participants or inducers of DC differentiation. During their maturation process, DC can migrate to various tissues. Some other dendritic cells are found in the thymus, spleen, lymph nodes and even in areas of the central nervous system. They reside in tissues in an immature stage and respond to various chemoattractants from inflammation sites. At the same time, they produce chemokines that recruit and activate macrophages, granulocytes, natural killer cells, and other immature DC. Once activated by microorganisms and inflammatory stimuli, DC undergo further maturation and migrate to the lymph nodes in search of antigen specific naïve T cells. During this migration, DC process the antigens they captured in the tissue areas, express costimulatory molecules, and become potent stimulators of T cells.

Antigen Processing

Intracellular Antigens

Viral proteins, or other components from intracellular pathogens in the cytoplasm, are reduced to peptides by proteasomes. Proteasomes are special protein complexes present in the cellular cytosol that have proteolytic function^{5,6}. Once the parasite proteins have been digested to small peptides, they are transported into the lumen of the endoplasmic reticulum via a heterodimeric integral membrane complex termed TAP (transporter associated with antigen processing). As the MHC class I heavy chain molecule is being folded in the ER, it is stabilized by a membrane-bound protein calnexin until the β 2-microglobulin (β 2m) subunit associates. When β 2m binds, calnexin is released and the MHC molecule associates with TAP by interacting with a TAP-associated protein tapasin and a chaperone protein calreticulin. The peptide brought in by TAP binds to the MHC molecule and completes the assembly of the complex. The MHC class I:peptide complex is released from tapasin and calreticulin, leaves the ER and is transported to the surface via the classical secretory pathway⁷⁻¹⁰ (Figure 1, a, b).

Figure 1

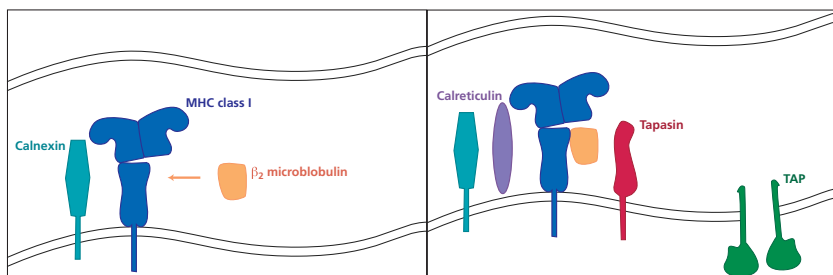


Figure 1a. Calnexin stabilizes Class I heavy chain prior to β_2 microglobulin arrival

Figure 1b. Class I heavy chain and β_2 microglobulin heterodimer forms complex with calreticulin, tapasin and TAP after calnexin release.

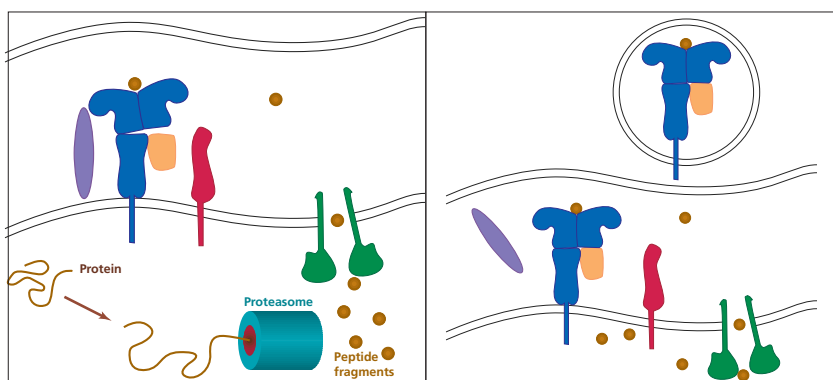


Figure 1c. TAP-delivered peptide binds to Class I heavy chain and forms mature MHC Class I molecule.

Figure 1d. Class I molecule is transported from the endoplasmic reticulum after dissociation from calreticulin, tapasin and TAP.

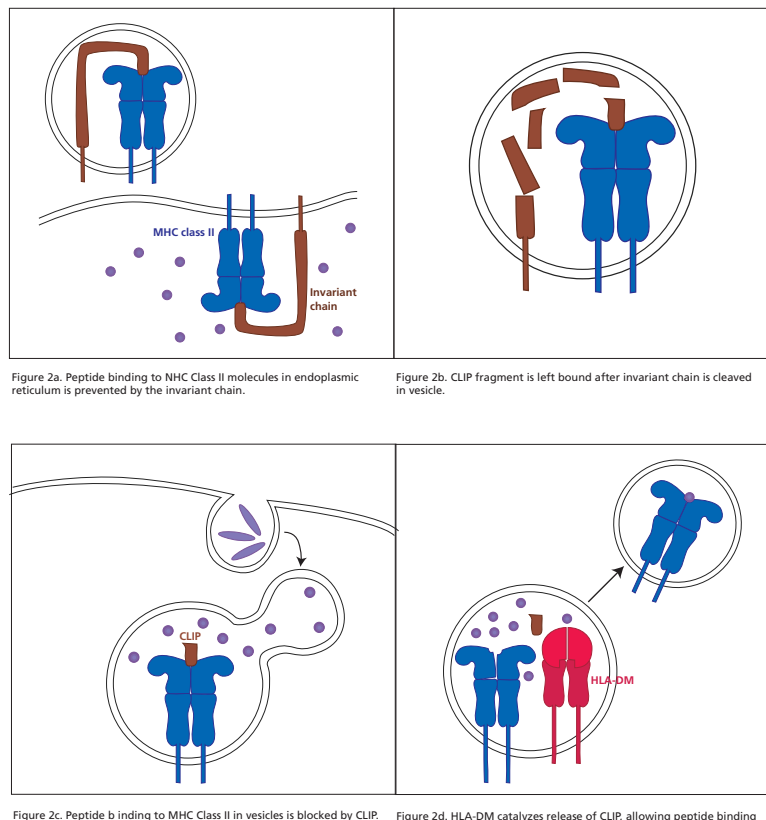
Extracellular Antigens

Antigens derived from extracellular parasites, like bacteria and other pathogens, are processed through a different pathway, which combines the internalization of extracellular antigen with the exocytosis of MHC class II molecules. Antigens are captured and internalized by macrophages, dendritic cells or B cells that will play the role of antigen presenting cells (APC). The extracellular material is engulfed through endocytosis (soluble molecules), or phagocytosis (bacterial particles, dead cells) and localizes to intracellular vesicles derived from the plasma membrane. As the vesicles mature, their interior becomes acidified due to the action of proton pumps and subsequent fusion with more late stage endocytic vesicles. The fusion event introduces proteolytic enzymes into the antigen-containing compartment. The subsequent proteolytic digestion generates a pool of peptides, some of which will have an affinity for MHC class II molecules. Before the MHC class II molecule is able to bind the peptide, it requires

special assembly and the participation of other molecules. The MHC molecule is initially assembled in the endoplasmic reticulum as a trimer of trimers. The three subunits consist of the alpha and beta antigenic peptide binding chains and a third chaperone like chain called invariant chain (Ii). The Ii chain serves at least three functions. In the classic definition of a chaperone, it facilitates the proper folding of the alpha and beta subunits and in its absence MHC class II molecules aggregate and do not leave the ER. A second function ascribed to Ii is its ability to prevent peptides in the endoplasmic reticulum from binding in the peptide-binding groove of MHC class II. This function thus prevents to one degree or another the binding of endogenous antigens onto this class of antigen presentation molecules and preserves the role of this group of molecules for presentation of exogenous antigens. The third function of Ii is to divert the MHC class II molecules from the classical secretory pathway into the endosomal/lysosomal system. This is accomplished by a

Dendritic Cells and Antigen Processing and Presentation *(continued from page 15)*

Figure 2



dileucine motif present in the cytoplasmic tail of this glycoprotein. This critical feature of Ii allows newly biosynthesized MHC class II molecules to intersect and fuse with vesicles containing internalized antigens. Many of the same proteases in endocytic vesicles responsible for digesting antigenic proteins into peptides also digest the invariant chain on MHC class II leaving a small peptide called class II-associated invariant chain peptide (CLIP) still bound to the MHC molecule. CLIP remains bound to prevent other peptides from binding the MHC molecule prematurely. CLIP is later released by the competitive displacement of antigenic peptides that have higher affinity for the binding groove of the class II molecule. This exchange is facilitated by another MHC encoded glycoprotein called HLA-DM. The loading is limited by a window of time as digestion of the Ii chain allows MHC class II to exit the endosomal lysosomal system and

vesicles containing MHC class II peptide complexes or MHC class II CLIP complexes are free to fuse with the plasma membrane¹¹⁻¹⁷ (Figure 2, a, b).

Antigen Presentation

Dendritic cells, monocytes and B cells fulfill the function of antigen presenting cells (APC). In skin, keratinocytes and Langerhans cells play the role of APC. These cells are specialized to present antigens on MHC class II molecules. Other cells like T cell neutrophils, basophils and NK cells, all have proteasomes and mainly express MHC class I constitutively. As cell types are susceptible to viral infections, this enables surveillance by CD8⁺ T lymphocytes.

MHC class I:peptide complexes are expressed on virus infected cells and recognized by CD8 T cells. MHC class II: peptide complexes are expressed on APC and recognized by CD4 T cells. Their recognition requires the participa-

tion of other molecules that trigger costimulatory or accessory signals. The participation of the CD4 or CD8 molecules in the recognition of the MHC:peptide complex is essential in this process. CD4 helps recognize the MHC class II: peptide complex and CD8 helps recognize peptides presented in the context of MHC class I¹⁸.

APCs express adhesion molecules (either constitutively or upon activation), which can strengthen the bonds between T cells and APC and lead to induction of accessory signals^{19,20}. A partial list of the adhesion molecules found on APC includes LFA-1 (CD11a/CD18), ICAM-1 (CD54), VCAM-1 (CD106) and LFA-3 (CD58).

APC can also express MHC Class I or Class II surface molecules that have an affinity for processed antigenic peptides and serve to present them to T cells. In addition to the adhesion molecules and MHC components, APC display costimulatory molecules, B7-1 (CD80), B7-2 (CD86), and probably a B7-X (still to be defined). Costimulatory molecules play a crucial role in determining T-cell responses to presented antigen²¹⁻²⁴.

The first phase (phase I) in the process of antigen presentation is the interaction of APC and lymphocytes via adhesion molecules. The second phase (phase II) involves the recognition of the antigen by the T-cell receptor (TCR). As the T cell recognizes an antigen through its TCR, it initiates signals which upregulate the expression of costimulatory molecules. Receipt of secondary signals provided by bound costimulatory molecules results in the production and secretion of cytokines, and expression of cytokine receptors on T cells. For example, T-helper cells produce interleukin-2 (IL-2) and upregulate IL-2 receptor following signaling by CD28 binding. On the APC side, this is driven by IFN- γ secretion by T cells then subsequent IL-12 production by APC's³⁶. Cytokines induce the upregulation of CD28 on T cells and CD86 on APC. As CD28 and CD86 (Figure 3) connect during phase III, other signals are triggered which induce the expression of

additional costimulatory molecules: CD80 on APC and CTLA-4 on T cells. The process is completed by proliferation of T cells (phase IV). As CD4⁺ T-helper cells undergo proliferation, they can evolve into either memory T cells or, in response to additional cytokines, into effector T cells of either T-helper type 1 (Th1) or T-helper type 2 (Th2). Alternatively, CD4⁺ T-helper cells can undergo activation induced cell death (AICD)²⁵⁻²⁹.

DC-SIGN and CMRF-56

BD Biosciences Pharmingen offers two new monoclonal antibodies specific for dendritic cells, CMRF-56, and

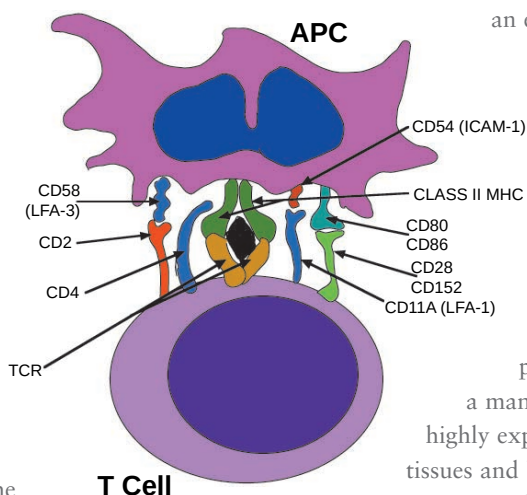
DC-SIGN (Figures 4). CMRF-56 reacts with

an early activation/differentiation antigen expressed on dendritic cells.

Circulating blood leukocytes and DC do not express the CMRF-56 antigen, but following either *in vitro* culture or activation of PBMC's, CMRF-56 antigen is expressed on DC and a subpopulation of CD19⁺ lymphocytes³⁰.

DC-SIGN, a type II membrane protein of approximately 44 kDa, with a mannose-binding, C-type lectin domain is highly expressed on dendritic cells in mucosal tissues and binds to ICAM-3 (CD50). Its sequence is identical to the HIV-1 envelope glycoprotein gp-120-binding C-type lectin and was renamed DC-SIGN³¹. Reports demonstrate that DC-SIGN binds to HIV-1 gp-120. DC-SIGN does not function as a viral receptor, but it allows dendritic cells to efficiently infect resting T cells expressing CD4 and chemokine receptors³². Reports also suggest that DC-SIGN enables the highly efficient migration of dendritic cells from blood into the tissues³³. It can interact with ICAM-2, which has a similar sequence as ICAM-3, and is abundantly expressed on vascular and lymphoid endothelium. Thus, DC-SIGN mediated dendritic cell rolling, transendothelial migration, and the ICAM-2 interaction is essential the specific migratory functions of dendritic cells.

Figure 3



Dendritic Cells and Antigen Processing and Presentation *(continued from page 17)*

Antigen processing and dendritic cell research reagents from BD Biosciences Pharmingen.*

Human Reagents

Cat. No.	Description	Clone	Isotype	Format	Size
555980	CLIP	CerCLIP	m IgG1	Purified	0.1 mg
555981	CLIP	CerCLIP	m IgG1	FITC	100 tests
551264	DC-SIGN	DCN46	m IgG1	FITC	100 tests
555982	HLA-DM	Map.DM1	m IgG1	Purified	0.1 mg
555983	HLA-DM	Map.DM1	m IgG1	PE	100 tests
559895	HLA-DO	DOB.L1	m IgG2b	Purified	0.1 mg
550020	HLA-DO	DOB.L1	m IgG2b	FITC Set	100 tests
550021	HLA-DO	DOB.L1	m IgG2b	PE Set	100 tests
551295	TAP1	TAP1.28	m IgG1	Purified	0.1 mg
551292	TAP2	TAP2.17	m IgG1	Purified	0.1 mg
550910	IP30	Map.IP30	m IgG1	Purified	0.1 mg
550915	IP30	Map.IP30	m IgG1	FITC Set	100 tests
Coming Soon	TAP2	TAP2.17	m IgG1	FITC Set	100 tests
Coming Soon	TAP2	TAP2.17	m IgG1	PE Set	100 tests
Coming Soon	Antibody Kit for Cultured Human Dendritic Cells†				
34224N	CD1a-FITC	HI149	mlgG ₁		0.05 mg
33075N	CD40-PE	5C3	mlgG ₁		0.05 mg
34235N	HLA-DR-PE	G46-6	mlgG _{2a}		0.05 mg
36794N	CD80-FITC	L307.4	mlgG ₁		0.05 mg
33404N	CD86-FITC	FUN-1	mlgG ₁		0.05 mg
36935N	CD83-PE	HB15e	mlgG ₁		0.05 mg
30545N	CD14-PE	M5E2	mlgG _{2a}		0.05 mg
33815N	Isotype Contr.-PE	MOPC-21	mlgG ₁		0.05 mg
33814N	Isotype Contr.-FITC	MOPC-21	mlgG ₁		0.05 mg
33035N	Isotype Contr.-PE	G155-178	mlgG _{2a}		0.05 mg

Mouse Reagents

Expression of cell surface markers on myeloid and lymphoid derived mouse spleen dendritic cells.

Cat. No.	Surface marker	Myeloid DC (spl.)	Lymphoid DC (spl.)
553843, 559871	CD1d	No	Yes
553646, 553042, 553052	CD4	Yes	No
553027, 553829	CD8a	No	Yes
553118	CD9	Yes	Yes
553118, 557453	CD11a	Yes	Yes
553308	CD11b	Yes	dim/neg
553799	CD11c	Yes	Yes
558743	CD13	Yes	Yes
553136	CD23	No	Yes
558777	CD24	Yes/low	Yes
553131, 558739	CD44	Yes	Yes
553250	CD54	Yes	Yes
553368, 553766	CD80	Low	Low
553689, 558784	CD86	Yes	Low
553326	CD102	Yes	Yes
see Catalog for individual haplotype	MHC Class I	Yes	Yes
see Catalog for individual haplotype	MHC Class II	Yes	Yes

† Panel of antibodies useful for detecting dendritic cells as well as an optimized protocol to generate cultured dendritic cells from peripheral blood mononuclear cells.

*All the BD Biosciences reagents highlighted in this article are "For research use only. Not for use in diagnostic or therapeutic procedures".

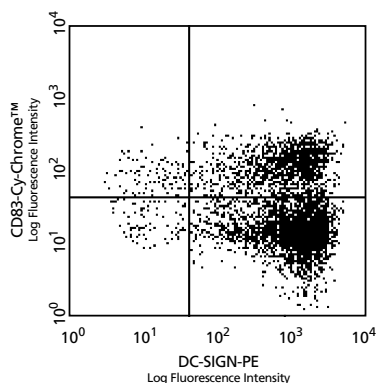


Figure 4. Profile of culture-derived dendritic cells (7 day) analyzed by flow cytometry.

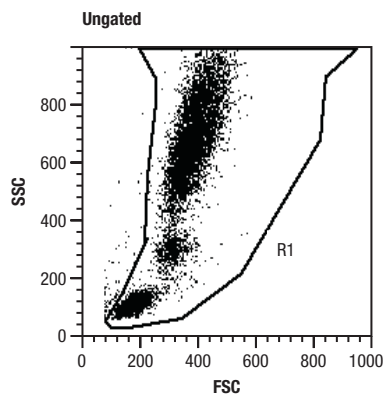


Figure 5. Human Peripheral Blood Dendritic Cell Identification. The following gating strategy applies to the 4-color assay. Whole blood, ungated SSC/FSC dot plot.

20% of total events are displayed.

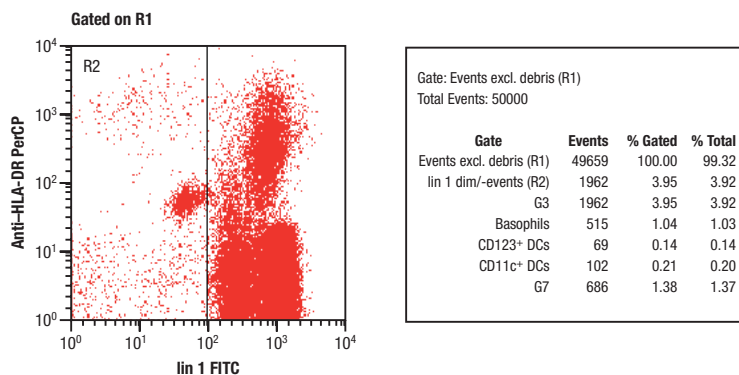


Figure 6. Whole blood, 4-color assay. Region R2 includes lin 1 dim and negative cells, such as DC populations, basophils, and eosinophils.

All R1 events are displayed.

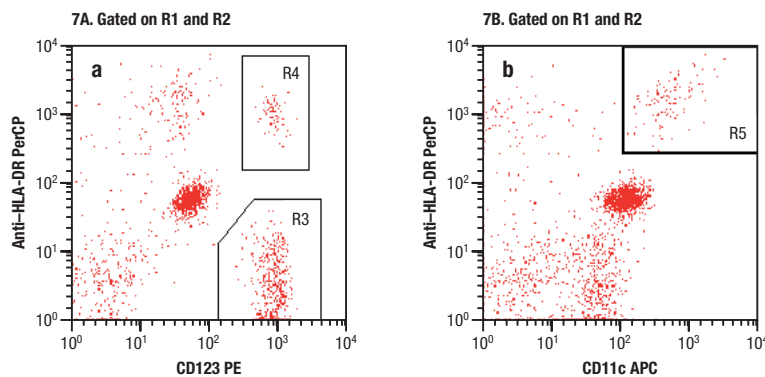


Figure 7. Whole blood, 4-color assay. Gated on events excluding debris and on lin 1dim/negative events, defined in logical gate G3.

7A. Region R3 defines basophils and region R4 CD123+ DCs.

7B. Region R5 defines CD11c+ DCs.

All R1 and R2 events are displayed.

Dendritic Cells and Antigen Processing and Presentation *(continued from page 19)*

Human Peripheral Blood Dendritic Cell Reagents from BD Biosciences Immunocytometry Systems*

CD123⁺ dendritic cells, CD11c⁺ dendritic cells, and basophils can be identified directly from peripheral blood. Based on a whole-blood flow cytometric assay, this system saves time and can identify multiple dendritic cell subsets in a single tube. With the first commercially available lineage cocktail, these rare cellular subsets can be identified without altering their function or phenotype^{34,35}. Learn more about these dendritic cell subsets and the advantages of dendritic cell identification using flow cytometry from our application note [\(Figure 5, 6, 7\)](#).

Peripheral Blood Dendritic Cells Revealed by Flow Cytometry (Please call Technical Services for your copy)^{34,35}.

Dendritic Cell Single Vial Reagents

Cat. No.	Antibody
340546	Lineage Cocktail 1 (lin 1) FITC (CD3,CD14,CD16,CD19,CD20,CD56)
340545	CD123 (Anti-IL-3R α) PE
347637	CD11c PE
340544	CD11c APC
347364	Anti-HLA-DR PerCP
340549	Anti-HLA-DR APC
349043	Mouse IgG1 PE
349053	Mouse IgG2a PE
340473	Mouse IgG2a APC

3-Color Dendritic Value Bundle

Cat. No.	Antibody
340566	Lineage Cocktail 1 (lin 1) FITC
	CD123 PE
	CD11c PE
	Anti-HLA-DR PerCP
	Mouse IgG1 PE
	Mouse IgG2a PE

4-Color Dendritic Value Bundle

Cat. No.	Antibody
340565	Lineage Cocktail 1 (lin 1) FITC
	CD123 PE
	CD11c APC
	Anti-HLA-DR PerCP
	Mouse IgG1 PE
	Mouse IgG2a APC

BD Biosciences recognizes the need in the scientific community for tools to accelerate dendritic cell research. We have continued, over the years, to search for antibodies that provide our customers with products to study the mechanisms of antigen processing and presentation. Please visit our website for new product announcements: www.bdbiosciences.com.

*All the BD Biosciences reagents highlighted in this article are "For research use only. Not for use in diagnostic or therapeutic procedures".

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NEW Products For Non-human Primate Research

By Enoc Hollemweguer Ph.D, Xiao-wei Wu, Joshua Cui, Melissa Heller, Sharon Sasaki, Feng-Jun Luan, Jay Dong, M.D, M.S, Zhang Chen, M.D.

To continue to provide the most complete line of reagents for Non-human Primate (NHP) research, BD Biosciences Pharmingen has developed new methodologies and improved on established protocols to detect cytokine responses in this animal model.

New NHP Reagents for Cytokine Flow Cytometry

We are proud to announce the availability of more anti-bodies that cross-react with NHP cytokines. These anti-bodies have been studied under various conditions in order to optimize our current protocols. Phorbol myristate acetate (PMA) and Ionomycin, or Lipopolysaccharide (LPS), are routinely used on NHP peripheral blood mononuclear cells (PBMC) to induce production of IFN- γ , IL-2, IL-4, IL-6, TNF- α as well as other cytokines. Superantigen staphylococcal enterotoxin B (SEB) can also be used to induce cytokine production and this resulted in a better IL-4 response than that induced by PMA and Ionomycin.

Briefly, NHP PBMC are stimulated with either PMA/Ionomycin or SEB in presence of Brefeldin A

or Monensin as protein transport inhibitors. Following incubation, the cells are fixed and permeabilized, then stained with the appropriate anti-cytokine antibodies. Anti-CD69 is also added to stain activated cells. After incubation with the antibodies, the cells are washed, resuspended and analyzed in a FACS Calibur.

The strategy for analysis offers options to compare cytokine production by activated (CD69⁺) or non-activated cells. The methodology works with either whole blood or PBMC. Using whole blood, Brefeldin A provides more optimal stimulation compared to Monensin, which may interfere with proper lysis of the sample.

New NHP Reagents for ELISA

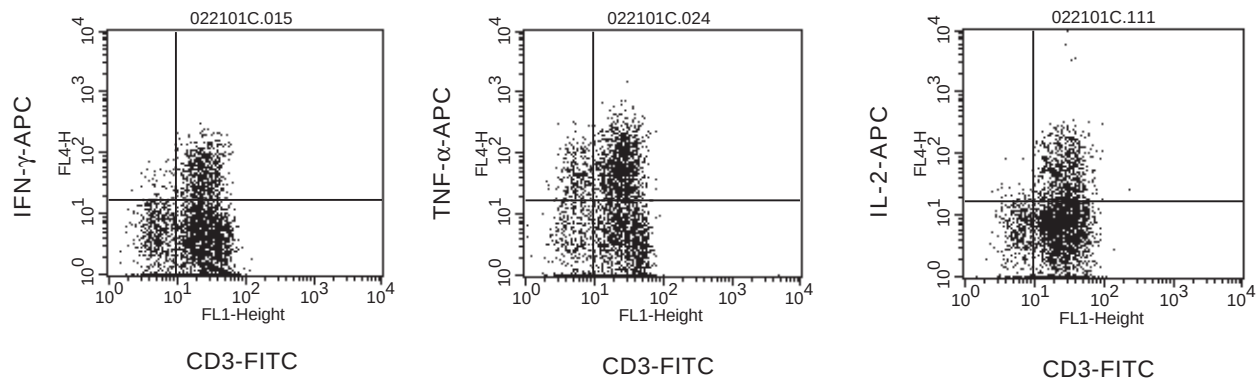
For a list of additional chemokine/cytokine antibodies that cross-react with monkey please see the article entitled BD OptEIA™ Monkey ELISA Sets for Rhesus, Cynomolgus, and Baboon Cytokine/Chemokine measurement, page 24 of this issue of Hotlines.

New NHP Reagents for Multi-color Analysis

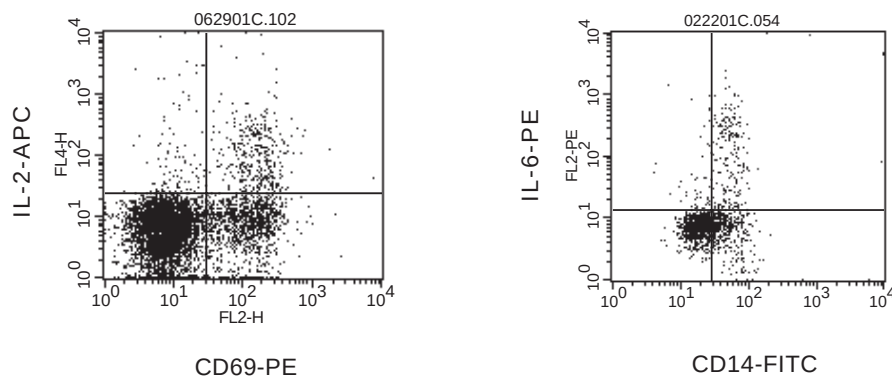
We have also developed new antibody combinations to simplify immunophenotyping of NHP leukocytes. These are three-color combinations that include CD45 or CD3 markers as gating tools. Below is a list of available multi-color reagents.

NHP Cytokine Flow Cytometry Reagents

New Cat. No.	Cat No.	Antibody	Clone	Format
557064	71205L	anti-IL-16	14.1	PE
557065	71215L	anti-IL-8	G265-8	PE
557066	71225L	anti-MCP-1	5D3-F7	PE
551532	71229L	anti-MCP-1	5D3-F7	APC
559361	71234L	anti-IL-2	MQ1-17H12	FITC
557067	71235L	anti-IL-2	MQ1-17H12	PE
551383	71239L	anti-IL-2	MQ1-17H12	APC
557068	71245L	anti-TNF- α	MAb11	PE
551384	71249L	anti-TNF- α	MAb11	APC
557069	71255L	anti-MIP1- α	11A3	PE
551533	71259L	anti-MIP1- α	11A3	APC
557070	71265L	anti-RANTES	2D5	PE
557074	71275L	anti-IFN- γ	4S.B3	PE
551385	71279L	anti-IFN- γ	4S.B3	APC
551473	71895L	anti-IL-6	MQ2-6A3	PE
551474	71899L	anti-IL-6	MQ2-6A3	APC
551774	71985L	anti-IL-4	8D4-8	PE



Profile of PMA/ionomycin-stimulated PBMC of cynomolgus macaque



Profile of SEB-stimulated
PBMC of rhesus macaque

Profile of LPS-stimulated peripheral
blood monocytes of rhesus macaque

Current Multi-color Reagents

New Cat. No.	Cat. No.	Description	Size
551070	7179CL	CD3-FITC/CD4-PE/CD45-PerCP	50 Tests
551069	7180CL	CD3-FITC/CD8-PE/CD45-PerCP	50 Tests
551068	7181CL	CD3-FITC/CD20-PE/CD45-PerCP	50 Tests
551067	7182CL	CD3-FITC/CD16-PE/CD45-PerCP	50 Tests

The data associated with these studies will be presented at the upcoming 19th Annual Symposium on Non-human Primate Models for AIDS, San Juan, Puerto Rico, November 7 - 10, 2001.

BD Biosciences Pharmingen appreciates the input and contribution of our valued customers as we continue to expand the Non-Human Primate reagent offering.

BD OptEIA™ Monkey ELISA Sets for Rhesus, Cynomolgus, and Baboon Cytokine/Chemokine measurement

By Feng-Jun Luan, Sharon Sasaki, Edward Morgan, Ph.D

The study of non-human primate (NHP) immune function is critical for the development of therapeutic and diagnostic reagents for human disease. The lack of antibody-based reagents specific for NHP has historically been problematic for researchers. BD Biosciences Pharmingen is proud to introduce a new panel of BD OptEIA™ ELISA Sets that can be used for cytokine/chemokine measurement in Rhesus, Cynomolgus, and Baboon serum, plasma, and cell culture supernatant samples.

Cross-Reactivity

We have analyzed multiple (n=28) anti-human specific antibody pair combinations (cytokines/chemokines) for their cross-reactivity with NHP. Eight anti-human cytokine ELISA pairs and one chemokine ELISA pair were found to cross-react with NHP at significant levels (Table 1). These results indicate that the utilization of cross-reactive antibodies is important for the measurement of cytokines/chemokines in NHP culture supernatants, serum, and plasma. Thus, cytokine/chemokine production in NHP disease models may parallel human levels, and relative values can be obtained in an ELISA-based system.

Correlation between BD OptEIA™ recombinant standard and NHP samples

To demonstrate that NHP samples can be accurately measured against a human recombinant standard, cell culture supernatants from Rhesus, Cynomolgus, and Baboon were serially diluted and run in parallel with the serially diluted BD OptEIA™ recombinant standard (Figure 1).

The parallel slopes of the three species of primates and the BD OptEIA™ recombinant human TNF- α standard indicate that this assay accurately measures relative levels of TNF- α . Similar parallelism has been demonstrated with the other eight cross-reactive antibody pairs.

BD OptEIA™ ELISA Sets contain sufficient matched, pre-titered reagents for twenty 96-well ELISA plates:

- Capture antibody
- Biotinylated Detection antibody
- Recombinant standard
- Avidin-horseradish peroxidase (HRP)

Table 1. Native Monkey Cytokine Values in BD OptEIA™ ELISA Sets

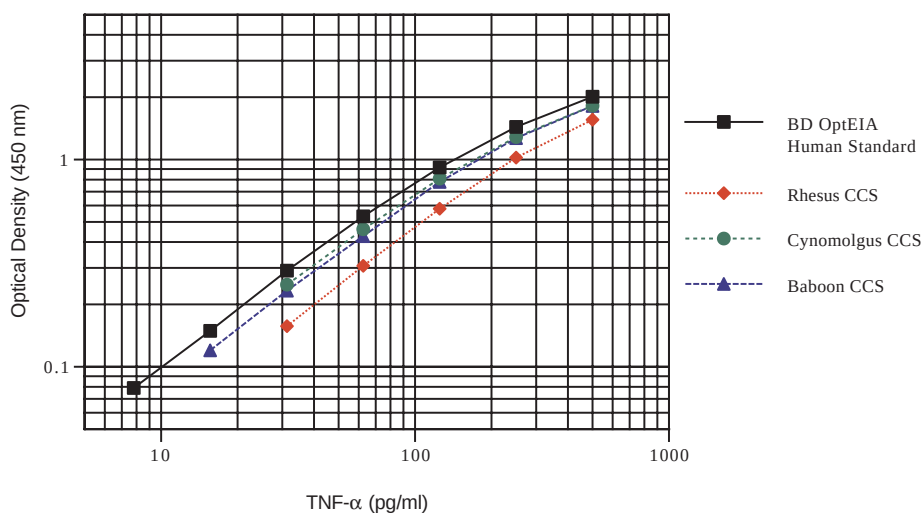
Human Ab Pairs	Rhesus CCS (units/ml)*	Cynomolgus CCS (units/ml)*	Baboon CCS (units/ml)*
IFN- γ	1,737.7	1,234.8	2,548.1
IL-2	22,397.3	5,688.9	8,272.8
IL-4	203.4	616.2	1146.4
IL-5	301.7	1,215.9	2133.7
IL-6	2109.7	3703.4	835.9
IL-8	54,190.2	91,682.6	30,193.2
MCP-1	14,982.2	15,640.0	9,092.9
TNF- α	599.2	1,793.0	830.7
IL-1 β	806.1	1862.9	29.1

Note 1: The cytokine/chemokine values are generated against human recombinant protein standards.

Note 2: In the BD OptEIA™ Monkey ELISA Sets, 1 unit = 1 picogram of recombinant cytokine/chemokine standard.

* The NHP samples were cell culture supernatants from NHP PBMC (2×10^6 cells/ml) that were cultured in IMDM plus 10% FBS and stimulated with PMA at 50 ng/ml and ionomycin at 0.5 μ g/ml for 16 hours (IL-1 β stimulation was with LPS plus monensin for 16 hours).

Figure 1. Parallelism of Rhesus, Cynomolgus, Baboon Cell Culture Supernatant in Monkey TNF- α OptEIA ELISA Set



Note: These curves depict the optical densities of cell culture supernatant samples when diluted in the range of the BD OptEIA™ recombinant human TNF- α standard curve. The quantitation of these samples is not reflected in this figure.

Product listing

New Cat. No.	Cat. No.	Description
551492	2750KI	Monkey IFN- γ BD OptEIA™ ELISA Set
551493	2751KI	Monkey TNF- α BD OptEIA™ ELISA Set
551494	2752KI	Monkey IL-2 BD OptEIA™ ELISA Set
551495	2753KI	Monkey IL-4 BD OptEIA™ ELISA Set
551496	2754KI	Monkey IL-6 BD OptEIA™ ELISA Set
Coming soon. Please inquire		Monkey IL-5 BD OptEIA™ ELISA Set
Coming soon. Please inquire		Monkey IL-8 BD OptEIA™ ELISA Set
Coming soon. Please inquire		Monkey MCP-1 BD OptEIA™ ELISA Set

NEW BD OptEIA™ CL Chemiluminescent ELISA Kits

By Sharon Sasaki, Susan Chambers, Feng-Jun Luan

Broader Assay Range, Superior Low-end Sensitivity

Chemiluminescence ELISA systems provide a broader dynamic assay range, superior low-end sensitivity, and a faster protocol than the conventional colorimetric methods. The new BD OptEIA™ CL Chemiluminescent ELISA Kits utilize a luminol-based chemiluminescent substrate and enhancer that result in rapid kinetic light output and high signal intensity. The specifically fragmented antibodies and diluents used in the BD OptEIA™ CL Kits enable quantitation of cytokines and other soluble proteins in serum, plasma, or cell culture supernatant samples.

The BD OptEIA™ CL Kits have been validated on the new BD Monolight 3096 Microplate Luminometer, an easy to use, ultra-sensitive, photon-counting luminometer (see *Hotlines*, Spring 2001).

The Human IFN- γ BD OptEIA™ CL Kit provides a dynamic assay range of 2.4 to 7,500 pg/ml. The standard curve is as shown in **Figure 1**. The sensitivity (minimum detectable dose) of IFN- γ was found to be 1.4 pg/ml.

BD OptEIA™ CL Assay Protocol:

1. 50 μ l ELISA Diluent per well.
2. 100 μ l standard or sample per well.
Incubate 2 hours at room temperature, wash.
3. 100 μ l Working Detector per well.
Incubate 1 hour at room temperature, wash.
4. 50 μ l Substrate (mixture of A+B) per well.
5. Read plate using BD Monolight 3096 Microplate Luminometer (**Figure 2**).

BD OptEIA™ CL Kit Includes:

- One Pre-coated 96-well Plate
- Lyophilized Standards: 3 vials
- Detection Antibody
- Avidin-HRP Conjugate
- ELISA Diluent
- Standard/Sample Diluent
- Wash Concentrate
- BD OptEIA™ CL Substrate A
- BD OptEIA™ CL Substrate B

New Cat No.	Cat. No.	BD OptEIA™ CL Chemiluminescent ELISA Kit
551501	2755KK	Human IFN- γ
551502	2756KK	Human TNF- α
551794	2759KK	Human IL-2
Coming soon. Please inquire		Human IL-4

New Cat. No.	BD Pharmingen™ Instruments
551280	BD Monolight 3096 Microplate Luminometer
551391	Injector Unit (2 injectors) for Monolight 3096
551393	BD Monolight 3096 with Injector Unit

Figure 1. BD OptEIA™ CL Human IFN- γ Chemiluminescent ELISA Kit

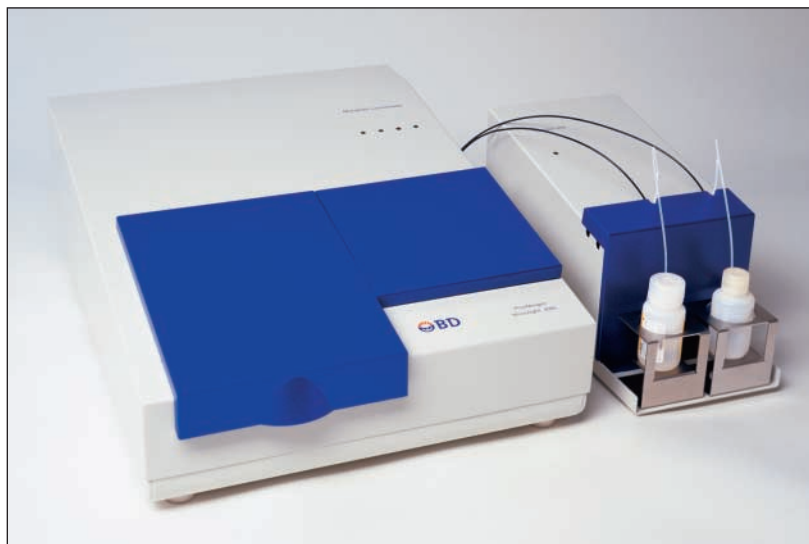
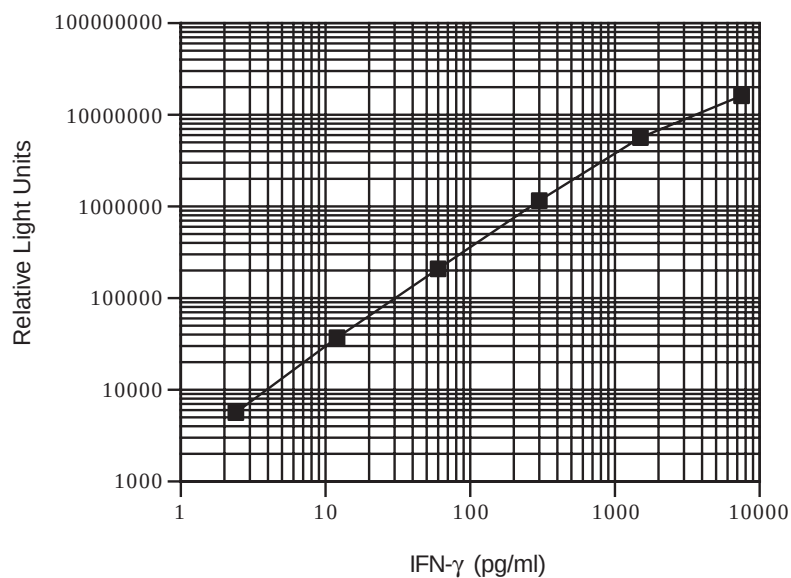


Figure 2. The BD Monolight 3096 Microplate Luminometer for the detection of bio- and chemiluminescence provides exquisite sensitivity and broad dynamic range for all luminescent assays in a 96-well format. Alone, or in combination with the Injector Unit, the Monolight 3096 is a powerful system capable of performing virtually any injection and measurement protocol, with single or dual measurement modes.

BD Biosciences Proteomics - Protein Discovery and Beyond

By Tony Ward

With the genomics era rapidly transitioning to a functional genomic and proteomic era, the importance of understanding relative protein expression or profiling is increasingly recognized as a critical component of the researcher's tools. In addition to comprehensive expression analysis, the integration of established biology remains an ongoing challenge, which we are gearing up to meet. It's always been critical to be able to tie the latest advances back to what has been done in the past. As a provider of tools that support the traditional methods of discovery, we also know what is important to keeping a clear focus on what will be needed going forward.

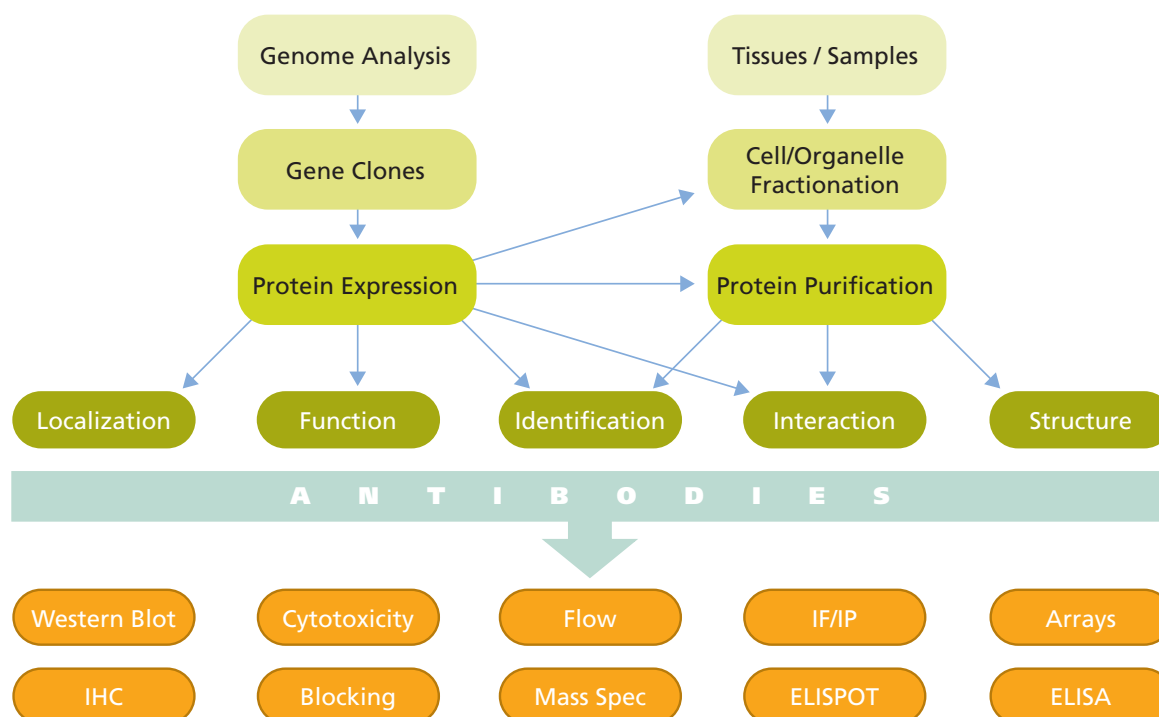
To meet our goal of providing leading edge reagents, we have licensed phage display technologies to augment our internal development and clone licensing approaches. This

combination of approaches will allow us not only to provide products to the research community, but also to be a content provider to industry supporting other platforms, such as protein microchip technology.

Across BD Biosciences, we offer solutions to help you get to where you want to go, whether it involves protein expression, purification, interaction, profiling, localization or function. The figure below describes how our product areas can help you in your proteomics work.

References:

1. Fialka L. et al (1999) J. Biol. Chem. 274, 26233-39; Meresse S. et al (1997) Electrophoresis 18, 2682-8; Boeck G. et al (1997) Trends in Cell Biology 7, 499-503
2. Kausch A.P. et al (1999) BioTechniques 26,336-343.



BD Biosciences Proteomics Product List

Protein Expression

Description	Size	Cat. No.
6xHis Monoclonal Antibody	200 µg	8916-1
6xHN Polyclonal Antibody	200 µl	8940-1
Cre recombinase	20 rxn	8480-1
Creator Acceptor vector kit	each	K1690-1
Creator pDNR-DUAL cloning kit	each	K1677-1
Creator PROTet 6xHN Bacterial Expression System	each	K1676-1
Creator RevTet Off System	each	K1674
Creator RevTet ON System	each	K1675
Creator SMART cDNA Libraries	each	Inquire
Creator SMART cDNA Library construction	each	K1260-1
Creator-Compatible PROTet 6xHN Bacterial Expression System	each	K1676-1
HAT Polyclonal Antibody	100 µl	8909-1
pDNR-LIB	each	6339-1
pHAT20 Vector	20 µg	8921-1
pLP-CMV-Myc Acceptor Vector	20 µg	6351-1
pLP-EGFP-C1 Acceptor Vector	20 µg	6343-1
pLP-EGFP-C1 Acceptor Vector	20 µg	6342-1
pLP-EYFP-C1 Acceptor Vector	20 µg	6341-1
pLP-GADT7 Acceptor Vector	20 µg	6349-1
pLP-GBKT7 Acceptor Vector	20 µg	6350-1
pLP-IRES2-EGFP Acceptor Vector	20 µg	6345-1
pLP-IRESneo Acceptor Vector	20 µg	6346-1
pLP-LNCX Acceptor Vector	20 µg	6344-1
pLP-PROTet-6xHN Acceptor Vector	20 µg	6352-1
pLP-PROTet-6xHN Acceptor Vector	20 µg	6352-1
pLP-RevTRE Acceptor Vector	20 µg	6347-1
pLP-TRE2 Acceptor Vector	20 µg	6348-1
PROTet 6xHN Bacterial Expression System	each	K1628-1

Protein Purification

Description	Size	Cat. No.
TALON Metal Affinity Resin	10 ml	8901-1
6xHis mAb-HRP Conjugate	100 µl	8906-1
CellThru 10-ml Disposable	20 cols.	8915-1
CellThru 2-ml Disposable	50 cols.	8914-1
TALON 2 ml Disposable Gravity Column	50 cols.	8903-1
TALON Buffer Kit	each	K1252-1
TALON CellThru 10	10 ml	8910-1
TALON CellThru 100	100 ml	8910-2
TALON Metal Affinity	25 ml	8901-2
TALON Metal Affinity	100 ml	8901-3
TALON Metal Affinity	250 ml	8901-4
TALON Purification Kit	each	K1253-1
TALON Superflow Metal	25 ml	8908-1
TALON Superflow Metal	100 ml	8908-2

Protein Interactions

Protein-Protein

MATCHMAKER Products*	Size	Cat. No.
Mammalian MATCHMAKER Two-Hybrid	each	K1602-1
MATCHMAKER BioSensor Kit	5 plates	K1616-1
MATCHMAKER Library Construction & Screening Kit	5 rxns	K1615-1
MATCHMAKER One-Hybrid System	each	K1603-1
MATCHMAKER Two-Hybrid System 3	each	K1612-1
pCMV-Myc & pCMV-HA Vector Set	each	K6003-1
pGADT7 AD Vector	20 µg	K1612-A

* The MATCHMAKER BioSensor Kit is covered by U.S. Patent #5,567,598, and patents pending

BD Biosciences Proteomics Product List *(continued from page 29)*

Protein Interactions *(continued)*

pGBKT7 DNA-BD Vector	20 µg	K1612-B
pLP-GADT7 AD Acceptor	20 µg	6349-1
pLP-GBKT7 DNA-BD Acceptor	20 µg	6350-1

Protein-DNA

Mercury TransFactor Kits	Size	Cat. No.
Mercury TransFactor c-Jun Kit	96 rxns	K2061-1
Mercury TransFactor NFATc1 Kit	96 rxns	K2060-1
Mercury TransFactor NFkB p50 Kit	96 rxns	K2058-1
Mercury TransFactor Profiling Kit - Inflammation 1	96 rxns	K2062-1
Mercury TransFactor STAT1	96 rxns	K2059-1

Confirmation

Living Colors Vectors	Size	Cat. No.
pd1EGFP-N1 Vector	20 µg	6073-1
pd2ECFP-N1 Vector	20 µg	6911-1
pd2EGFP-N1 Vector	20 µg	6009-1
pd2EYFP-N1 Vector	20 µg	6913-1
pd4EGFP-N1 Vector	20 µg	6072-1
pDsRed2-C1 Vector	20 µg	6974-1
pDsRed2-N1 Vector	20 µg	6973-1
pECFP-C1 Vector	20 µg	6076-1
pECFP-N1 Vector	20 µg	6900-1
pEGFP-C Vector Set	each	K6000-1
pEGFP-C1 Vector	20 µg	6084-1
pEGFP-C2 Vector	20 µg	6083-1
pEGFP-C3 Vector	20 µg	6082-1
pEGFP-N Vector Set	each	K6001-1
pEGFP-N1 Vector	20 µg	6085-1
pEGFP-N2 Vector	20 µg	6081-1
pEGFP-N3 Vector	20 µg	6080-1
pEYFP-C1 Vector	20 µg	6005-1
pEYFP-N1 Vector	20 µg	6006-1
pLEGFP-C1 Retroviral Vector	20 µg	6058-1
pLEGFP-N1 Retroviral Vector	20 µg	6059-1

Protein Profiling

Expression Profiling	Size	Cat. No.
Antibody Arrays: over 350 spotted proteins	each	Coming Soon
Cytometric Bead Array - Human Th1/Th2 Cytokine Kit	each	550749
Cytometric Bead Array - Human Th1/Th2 Cytokine II (contains IL-6)	each	551809
Cytometric Bead Array - Mouse Cytokine Th1/Th2	each	551287

Localization

Living Colors Vectors <i>(see above)</i>		
Multiple Tissue Arrays	Size	Cat. No.
Human MTA-Formalin Fixed	each	551253
Human MTA-Zinc (Formalin Free)	each	551255
Mouse MTA-Formalin Fixed	each	551260
Mouse MTA-Zinc (Formalin Free)	each	551261
Rat MTA-Formalin Fixed	each	551257
Rat MTA-Zinc (Formalin Free)	each	551259
Human Protein Medleys	Size	Cat. No.
Adrenal Gland	100 µl	7818-1
Aorta	100 µl	7809-1
Bladder	100 µl	8260-1

Localization (continued)

Human Protein Medleys	Size	Cat. No.
Brain	100 µl	7800-1
Brain, amygdala	100 µl	7821-1
Brain, cerebellum	100 µl	8251-1
Brain, cerebral cortex	100 µl	7827-1
Brain, frontal lobe	100 µl	7822-1
Brain, hippocampus	100 µl	7823-1
Brain, hypothalamus	100 µl	7824-1
Brain, temporal	100 µl	7825-1
Brain, thalamus	100 µl	7826-1
Brainstem	100 µl	8250-1
Cecum	100 µl	8259-
Duodenum	100 µl	8255-1
Esophagus	100 µl	8254-1
Fetal Brain	100 µl	8265-1
Fetal Kidney	100 µl	8266-1
Fetal Liver	100 µl	8267-1
Fetal Lung	100 µl	8268-1
Heart	100 µl	7801-1
Ileum	100 µl	8257-1
Jejunum	100 µl	8256-1
Kidney	100 µl	7802-1
Liver	100 µl	7805-1
Lung	100 µl	7803-1
Lymph Node	100 µl	7819-1
Mammary Gland	100 µl	8269-1
Ovary	100 µl	7807-1
Placenta	100 µl	7806-1
Prostate	100 µl	8261-1
Skeletal Muscle	100 µl	7804-1
Small Intestine	100 µl	7812-1
Smooth Muscle	100 µl	8258-1
Spinal Cord	100 µl	7828-1
Spleen	100 µl	7813-1
Stomach	100 µl	7814-1
Testis	100 µl	7808-1
Thyroid	100 µl	7817-1
Tongue	100 µl	8253-1
Trachea	100 µl	8252-1
Uterus (no endometrium)	100 µl	8263-1
Uterus	100 µl	8262-1
Vagina	100 µl	8264-1

Function/State

Status-Specific Antibodies	Size	Cat. No.
Over 1,000 antibodies to measure and stimulate cellular pathways including phospho-specific mAbs.		See catalog for details

Sample Preparation for Organelles

Try our antibodies to purify organelles for protein identification by 2D gel electrophoresis, mass spectrometry, or to assess the purity of your organelle preparations. Organelle specific antibodies and organelle reactive dyes have been used to assess the purity of organelle preparations and for further purification by FACS sorting¹ and magnetic particle based selection². The selected antibodies have been shown to work in immunofluorescence assays, and may be suitable for such applications.

Caveolae	Size	Cat. No.
Caveolin	50 or 150 µg	C13630
Caveolin 1	50 or 150 µg	C13620

BD Biosciences Proteomics Product List *(continued from page 31)*

Sample Preparation for Organelles *(continued)*

Caveolae	Size	Cat. No.
Caveolin 1	50 or 150 ug	C36220
Caveolin 1	50 or 150 ug	C37120
Caveolin 1	50 or 150 ug	C43420
Caveolin 2	50 or 150 ug	C57820
Caveolin 3	50 or 150 ug	C38320
Flotillin-1	50 or 150 ug	F65020
Flotillin-2/ESA	50 or 150 ug	E35820
Phospho-Caveolin (Y14)	50 or 150 ug	C91520
Endosomes	Size	Cat. No.
EEA1	50 or 150 ug	E41120
Endoplasmic Reticulum	Size	Cat. No.
BiP/GRP78	50 or 150 ug	G73320
ERp72	50 or 150 ug	E72920
SQ5	50 or 150 ug	S10120
SRP54	50 or 150 ug	S71420
Golgi	Size	Cat. No.
Bet1	50 or 150 ug	B32320
Cab45	50 or 150 ug	C77720
GM130	50 or 150 ug	G65120
GMAP-210/Trip230	50 or 150 ug	G10020
Golgin-84	50 or 150 ug	G93720
GS15	50 or 150 ug	G72420
GS27	50 or 150 ug	G76220
GS28	50 or 150 ug	G83820
p115	50 or 150 ug	P67420
p230 trans Golgi	50 or 150 ug	G88620
TGN38	50 or 150 ug	T69020
Lysosomes	Size	Cat. No.
Lamp-1	50 or 150 ug	L76620
SNX1	50 or 150 ug	S95620
SNX2	50 or 150 ug	S90020
Cathepsin L	50 or 150 ug	C78820
Mitochondria	Size	Cat. No.
Aralar	50 or 150 ug	A78520
ASS	50 or 150 ug	A10020
Hsp60	50 or 150 ug	H99020
Mcl-1	50 or 150 ug	M54020
MCM	50 or 150 ug	M17520
Metaxin	50 or 150 ug	M16020
Tim23	50 or 150 ug	T85720
Nucleus	Size	Cat. No.
CHD3	50 or 150 ug	C25020
Coilin	50 or 150 ug	C28020
Exportin-1/CRM1	50 or 150 ug	E11620
Exportin-t	50 or 150 ug	E95720
Karyopherin alpha/Rch-1	50 or 150 ug	R43020
Karyopherin beta	50 or 150 ug	K48020
Ki-67	50 or 150 ug	K72820

Sample Preparation for Organelles (*continued*)

Nucleus	Size	Cat. No.
MCM6	50 or 150 ug	M13120
Mitosin	50 or 150 ug	M62420
NDP52	50 or 150 ug	N77920
No55	50 or 150 ug	N83420
NTF2	50 or 150 ug	N42420
Nucleoporin p62	50 or 150 ug	N43620
NuMA	50 or 150 ug	N48120
Nup88	50 or 150 ug	N22220
PCNA	50 or 150 ug	P56720
Pericentrin	50 or 150 ug	P14120
Psme3/PA28-gamma	50 or 150 ug	P83620
Ran	50 or 150 ug	R32620
RanBP1	50 or 150 ug	R61720
TAP	50 or 150 ug	T10120
Transportin	50 or 150 ug	T57720
LAP2	50 or 150 ug	L74520
LAP1	50 or 150 ug	L78220
Peroxisomes	Size	Cat. No.
PECI	50 or 150 ug	P15320
PEX19	50 or 150 ug	P10620
PEX5	50 or 150 ug	P10420
Synaptic Vesicles	Size	Cat. No.
Synaptotagmin	50 or 150 ug	S39520
Synaptogyrin	50 or 150 ug	S52520

BD Biosciences and Dyax Corp. Announce Research Products and Proteomics Alliance

Franklin Lakes, NJ and Cambridge, MA (June 13, 2001) – BD Biosciences (BDB), a division of BD (Becton, Dickinson and Company) (NYSE:BDX), and Dyax Corp. (Nasdaq: DYAX) today announced the establishment of a strategic relationship for the discovery of antibodies using Dyax's proprietary phage display technology. Under the terms of the collaboration, BDB has obtained rights to antibodies identified using Dyax's proprietary human antibody library and screening technology.

BDB will have the exclusive right to market the Dyax antibodies as research products to the life science market. Dyax has retained all rights to use these antibodies in the therapeutic field. Each company has the right to collaborate with others to develop proteomics products using these antibodies.

According to Deborah J. Neff, President, BD Biosciences, "Our partnership with Dyax is a fundamental step to position BDB to be a leader in the rapidly emerging field of proteomics. BDB, through its Pharmingen, Transduction Laboratories and Immunocytometry Systems product groups, already is a world leader in providing antibodies against cellular and intracellular markers in human, mouse and other species. Dyax's phage display technology will accelerate the generation of thousands of new antibodies significantly enhancing BDB's position as the partner of choice in proteomics."

"BDB is an ideal partner to drive the power of phage display technology in the research products and proteomics fields," said Henry Blair, Chairman and Chief Executive Officer of Dyax Corp. "This is another case of Dyax collaborating with a world leader to exploit the power of phage display in their market, generating both short term revenue and royalties for Dyax. Our corporate partnering strategy provides resources and focus to support Dyax's principal objective, the development of human therapeutic products."

BD BaculoGold™ Max-XP Serum-Free Insect Cell Medium

New Product Highlight



The genomic era of biomedical research has given rise to the birth of proteomics as a scientific discipline. Vital to the advancement of proteomic research are the ability to efficiently clone a gene into an expression system and the ability to maximize the expression of the target recombinant protein. For over twelve years, BD Biosciences Pharmingen has set the industry standard by supplying the highest quality reagents for analyzing gene expression, protein expression, and cellular response and interaction. We strive to maintain this position and are proud to be the leading supplier of premium reagents for generating recombinant proteins using the BD BaculoGold™ Baculovirus Expression Vector System. Our latest innovation comes in the form of our new BD BaculoGold™ Max-XP Serum-Free Insect Cell Medium (cat. no. 551411).

BD BaculoGold™ Max-XP is produced using a proprietary system, resulting in a metabolically-enhanced medium that far outperforms the competition. Experiments indicate that the production of functional recombinant protein in insect cell lines grown in BD BaculoGold™ Max-XP increases up to three-fold compared with insect cells grown in complete TNM-FH and other serum-free media. In addition, BD BaculoGold™ Max-XP is designed to be used in multiple cell lines and has been shown to optimize cell growth and recombinant protein production in *Drosophila melanogaster*, *Trichoplusia ni*, *Heliothis zea*, the *Spodoptera frugiperda* cell lines Sf9 and Sf21, and more.

BD BaculoGold™ Max-XP is ideal for quick, direct adaptation of your insect cells cultured in serum-supplemented media or in serum-free media. If you're currently using either our BD BaculoGold™ TMN-FH Insect Cell Medium or BD BaculoGold™ Protein-Free Medium, minimal adaptation is necessary. Typical results are 4 to 8 passages for complete adaptation. It must be noted, however, that some cell lines are more sensitive to media changes and will require more than 8 passages. (Please contact our Technical Service Department for assistance with either direct or sequential adaptation techniques.)

BD BaculoGold™ Max-XP promotes high cell density and optimum protein production, making it ideal for both large-scale industrial use and research-scale production. In addition, BD BaculoGold™ Max-XP's composition is precisely defined, which facilitates the purification of recombinant proteins and isolation of virus.

In summary, this metabolically-designed serum-free medium enhances the growth of many types of insect cells, amplifies recombinant protein production, and augments baculovirus yield.

Modern Tools for Detecting & Analyzing Apoptosis

By Lisa S. Stein, Ph.D, Michael J. Boyer, Ph.D,
Anissa Agadir, Hai Le, Dana Radman, Andy Castellanos,
and David George

The impact of apoptosis on various cellular processes underlies the basis for the tools that are available for detecting and analyzing apoptotic cells. This article focuses on applications for the assays most commonly used and accepted by the scientific community to determine whether cells are undergoing apoptosis. We will show data generated by scientists and research associates in our Cell Biology/Cell Signaling laboratory using BD Biosciences Pharmingen reagents.

Apoptosis signaling can be generally divided into receptor- and mitochondrial-mediated pathways. These pathways converge at a number of downstream points including the mitochondria, caspase activation, and substrate cleavage. Apoptosis is a highly conserved process; ergo, the majority of apoptosis assays can be used for a wide variety of model systems. In the examples presented here, apoptosis was induced either through the Fas receptor-mediated pathway using agonistic anti-Fas monoclonal antibodies, or through the mitochondrial-mediated pathway using staurosporine (a protein kinase inhibitor) or camptothecin (a topoisomerase I inhibitor).

When apoptosis is induced, procaspases are proteolytically cleaved and reassemble to form active heterotetrameric enzymes (caspases). Active caspase-3 has emerged as a powerful marker of cells undergoing apoptosis; its presence is considered to be diagnostic for apoptosis. Antibodies recognizing caspase-3 are useful tools for assessing its activation status (Figure 1). In this experiment, proteins from Jurkat T cell lysates were immunoprecipitated with monoclonal antibodies specific for either the active form of caspase-3 [lanes 1 and 3: clone C92-605, Cat. No. 68651G (recognizes the 17-22 kDa large subunit of active caspase-3)] or for both procaspase-3 (31 kDa) and active caspase-3 (17-22 kDa) (lanes 2 and 4: clone 19, Cat. No. C31720). This was followed by western blot with polyclonal antibodies recognizing both pro and active caspase-3 (Cat. No. 65906E). The results show that active caspase-3 is present in camptothecin-treated cell lysates (lanes 3 and 4), but not in control cell lysates (lanes 1 and 2),

which contain procaspase-3 only (lane 2). Therefore, we determine that only the camptothecin-treated lysate is apoptotic. Furthermore, these data show that procaspase-3 is also present in the apoptotic population (lane 4). This indicates that not all cells in the population were undergoing measurable apoptosis when the lysate was made. This is consistent with the dogma that cells in a population do not generally undergo apoptosis at the same time.

Apoptosis signaling results in the cleavage of multiple protein substrates en masse by active caspases, which leads to the loss of cellular structure and function, and ultimately results in cell death. Cleaved proteins are thus markers of apoptosis, and cleavage site-specific antibodies are emerging as particularly valuable tools for identifying apoptotic cells. PARP [Poly(ADP-ribose)polymerase], a 116 kDa nuclear chromatin-associated enzyme involved in DNA repair, is a classical example. It was the first molecule discovered to be cleaved by active caspases during apoptosis, and cleavage results in 85 kDa and 25 kDa fragments. In this experiment, HeLa cells were induced to undergo apoptosis with staurosporine. The fluorescent micrographs shown in Figure 2 indicate that cleavage site-specific PARP-FITC antibodies (polyclonal, Cat. No. 81114E) stain the staurosporine-treated (B, page 36), but not the control cells (A, page 36). A' and B' (page 36) represent phase contrast correlates of A and B respectively.

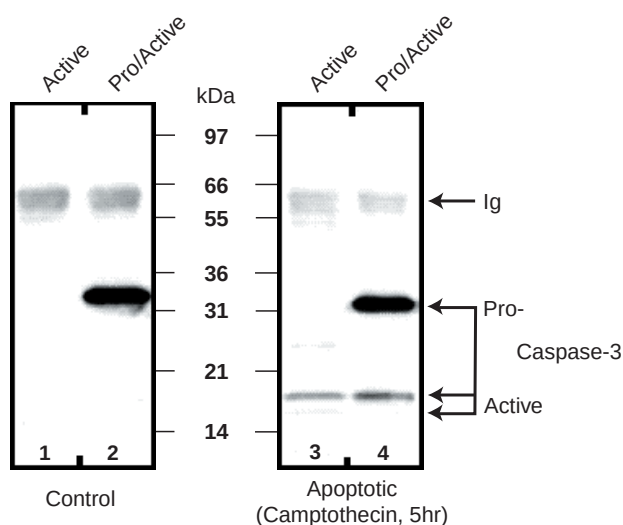


Figure 1. Caspase-3 Activation as a Marker of Apoptosis

Modern Tools for Detecting & Analyzing Apoptosis *(continued from page 35)*

Another measurable feature of apoptosis is the loss of plasma membrane asymmetry. In normal cells, membrane phospholipids are distributed asymmetrically between the inner and outer leaflets of the plasma membrane. For example, phosphatidylserine (PS), an aminophospholipid, is normally present in the inner leaflet of the plasma membrane. Early in apoptosis, before the loss of membrane integrity, PS translocates from the inner to the outer leaflet of the plasma membrane, exposing it to the external cellular environment at the surface of the plasma membrane. Annexin V is a 35-36 kDa, calcium-dependent, phospholipid-binding protein with a high affinity for PS and is used to indirectly monitor PS translocation. The Annexin V assay is perhaps the most widely used assay today, facilitated by the observation that PS translocation appears to be a universal apoptotic phenomenon. It has been detected in mammalian, insect, and plant cells under the action of most, if not all, triggers of apoptosis.

Although the Annexin V and Active Caspase-3 assays are based on different biological phenomena, they are both

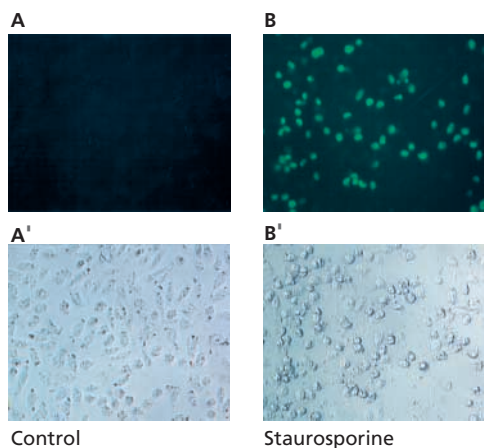


Figure 2. Immunofluorescence Micrographs of Cleaved PARP

used to identify cells that are in the early stages of apoptosis (Figure 3). Jurkat T cells were incubated with camptothecin for 0 to 4h, stained with either Annexin V-PE (Cat. No. 6900KK) or an Active Caspase-3 FITC monoclonal antibody (Cat. No. 6976KK), and analyzed by flow cytometry. Untreated samples were primarily negative for both markers, positive peaks were apparent at 0.5h, and the percentages of positive cells increased over the 4h

treatment. These results are also consistent with the dogma that apoptosis is a process that occurs over time and that not all cells in a population undergo apoptosis at once.

In contrast to Caspase-3 activation and PS exposure, DNA fragmentation occurs in the later stages of apoptosis and is typically measured using TUNEL (terminal deoxynucleotidyltransferase dUTP nick end labeling) assays such as APO-BRDU™ (Cat. No. 6576KK), which measures the incorporation of brominated dUTP (Br-dUTP) into the genome of apoptotic cells. In this study, Fas-induced Jurkat T cells were labeled with Annexin-V PE (Cat. No. 6900KK) or with APO-BRDU™ reagents. The flow cytometry results presented in Figure 4 indicate a significant increase in the percent of Annexin-V-positive cells, but not in Br-dUTP-positive cells, after 2h of incubation with Fas. At the 4h time point however, a significant increase in Br-dUTP-positive cells was observed. These results support the hypothesis that Annexin-V can detect cells at earlier stages of apoptosis than assays based on DNA fragmentation.

Assays measuring the mitochondrial membrane potential ($\Delta\psi$) are becoming popular for investigating the effects of apoptosis on the mitochondria. The dye JC-1 penetrates cells and is taken up into healthy mitochondria with polarized $\Delta\psi$, where it forms aggregates. JC-1 aggregates show a red spectral shift resulting in high levels of red fluorescence which is measured in the FL-2 channel (Figure 5). JC-1 does not accumulate in mitochondria with depolarized $\Delta\psi$ and remains in the cytoplasm as monomers, which do not have the red spectral shift. Both JC-1 aggregates and monomers exhibit fluorescence in the green end of the spectrum which is measured in the FL-1 channel. In the control cell population, JC-1 fluorescence is primarily seen in both the FL2 and FL1 channels (R1). In the staurosporine-treated sample there is a significant increase in the number of cells with lowered red fluorescence [FL2 (R2)], indicating $\Delta\psi$ depolarization.

In summary, although their underlying cellular mechanisms vary, all the assays discussed here are uniquely valuable for identifying cells undergoing apoptosis. The decision to select a particular assay(s) is an individual one, but often relies on a combination of the sample material available, the technique desired, and the cellular phenomena under examination.

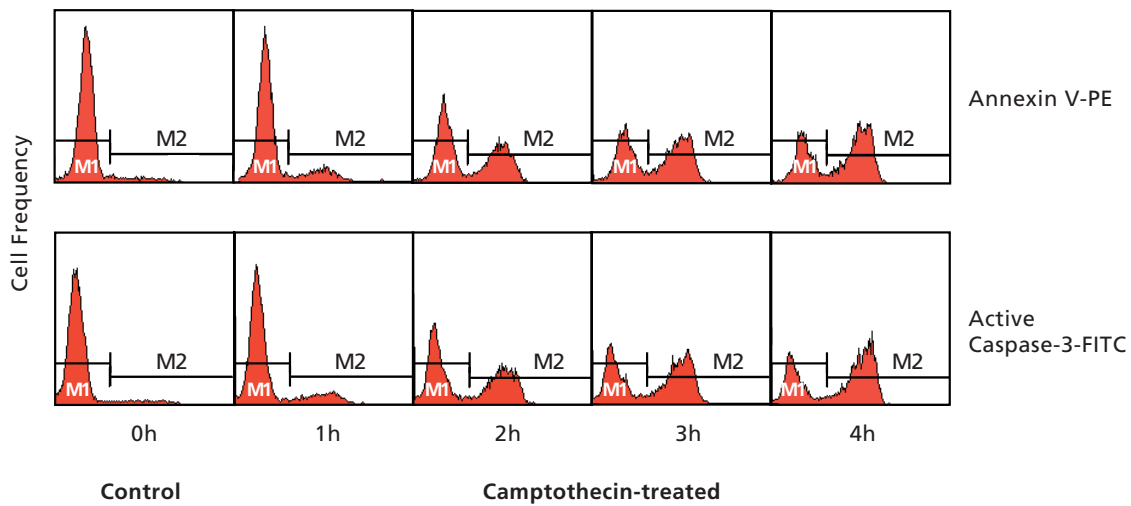


Figure 3. Comparison of Annexin-V and Active Caspase-3 Assays

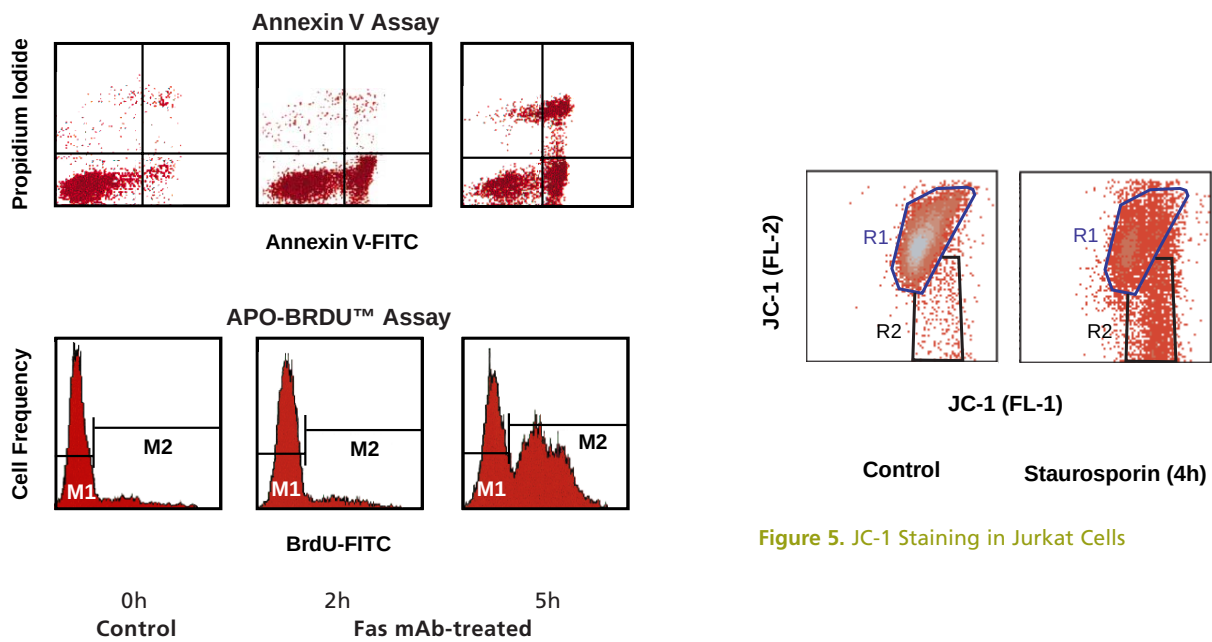


Figure 4. Comparison of Annexin-V and APO-BRDU™ Assays

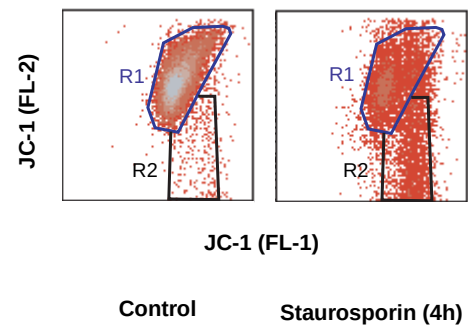


Figure 5. JC-1 Staining in Jurkat Cells

* APO-BRDU™ is a registered trademark of Phoenix Flow Systems.

Swine Natural Killer Cells

By Jennifer Bickel and Belen Ybarrondo

Natural Killer (NK) cells are large granular lymphocytes with non-adaptive non-MHC restricted cytotoxicity. They play an important role in resistance to tumor cells, virally infected cells and allogeneic grafts. NK cells also regulate the immune response through secretion of cytokines such as IL-2 and IFN- γ . Cells with NK activity have been identified in many different animal models, including swine. The swine immune system differs from other species in the existence of four peripheral lymphocyte populations defined by the expression of the CD4 and CD8 antigens (CD4⁺CD8^{lo}, CD4⁺CD8^{lo}, CD4⁺CD8^{hi}, CD4⁺CD8⁺) (Figure 1). The CD4⁺CD8^{lo} cells are involved in non-MHC-restricted lymphocyte functions associated with NK cells such as spontaneous cytolytic activity against tumor cells, generation of lymphokine activate killer (LAK) activity, and proliferative response against syngeneic leukocytes in mixed leukocyte reactions¹.

As the availability of various monoclonal antibodies raised against swine antigens has increased, a more comprehensive phenotypic characterization of swine NK cells has been performed. The swine NK cell population has been phenotypically characterized as CD2⁺ CD3⁻ CD4⁻ CD5⁻ CD6⁻ CD8^{dim} CD16⁺. Below, we briefly describe the phenotypic and functional characteristics of the swine NK cell population.

Swine CD16 is present on NK cells (CD4⁺CD8^{lo}) (Figure 2). CD16 is a low-affinity receptor for IgG and is also found on macrophages, monocytes, and granulocytes. The antigen recognized by the FcG7 monoclonal antibody has been identified as the swine Fc γ RIIIa (CD16) homologue². It interacts at the cell surface with a cytolytic trigger molecule on swine NK cells and neutrophils. The FcG7 monoclonal antibody induces and enhances cytotoxicity of NK cells and neutrophils against nucleated FcR⁺ target cells^{2,3,4}. Analysis of swine CD5 and CD6 antigens enables a further characterization of NK cells. The non-MHC-restricted cell-mediated cytotoxicity is associated with NK cells, which are CD4⁻ CD8^{lo} CD5⁻ CD6⁻ lymphocytes (Figure 3). T lymphocytes with MHC-restricted

cytotoxicity are found in the CD5⁺ and CD6⁺ populations^{1,5}. Swine CD2 is a membrane glycoprotein expressed on peripheral T lymphocytes and thymocytes. Swine CD2 is also present on NK cells (data not shown). It has been shown that depletion of CD2⁺ cells from PBL abolishes the NK activity⁶.

The swine is an important large animal model for a variety of immunological studies. Recently, the potential for swine to be xenogeneic organ donors to man has increased the need for further examination of the swine immune system. BD Biosciences Pharmingen is committed to expanding the swine product line to support your research. For new and upcoming pig reagents, please contact BD Biosciences Pharmingen Technical Services.

References:

1. Saalmuller. 1996. Differentiation between MHC-restricted and non-MHC-restricted porcine cytolytic T lymphocytes. *Immunology*; 88:238-246.
2. Kim, Y. 1994. Molecular cloning and identification of the porcine cytolytic trigger molecule G7 as a Fc γ RIIIa (CD16) homologue. *The Journal of Immunology*; 153: 2631-2641.
3. Kim, Y. 1995. Characterization of the cytolytic trigger molecules G7/PNK-E as a molecular complex on the surface of porcine phagocytes. *Cellular Immunology*; 161: 270-278.
4. Kim, Y. 1996. Identification of a unique porcine Fc γ RIIIa molecular complex. *Cellular Immunology*; 172: 92-99.
5. Saalmuller. 1994. Analyses of monoclonal antibodies reactive with porcine CD5. *Veterinary Immunology and Immunopathology*; 43: 237-242.
6. Yang, H. 1996. Phenotypic classification of porcine lymphocytes subpopulations in blood and lymphoid tissues. *Immunology*; 89: 76-83.

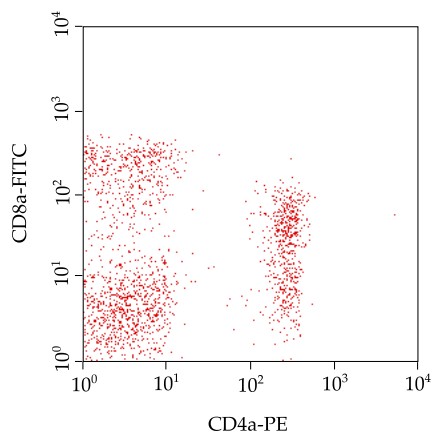


Figure 1. Swine peripheral lymphocytes were stained with anti-pig CD4a PE mAb 74-12-4 (Cat. No. 559586) and anti-CD8a FITC mAb 76-2-11 (Cat. No. 551303). For data analysis, a gate was set to include only viable lymphocytes.

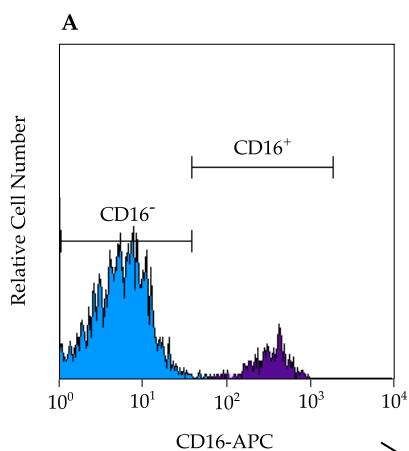
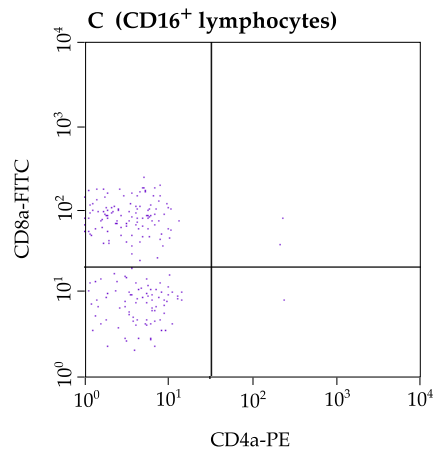
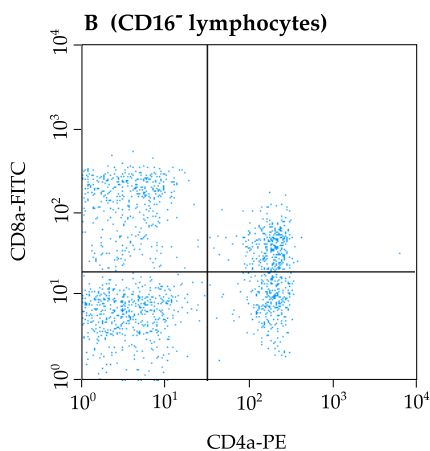


Figure 2. Swine peripheral blood was simultaneously stained with anti-pig CD4 PE, anti-pig CD8 FITC, and biotinylated anti-pig CD16 mAb FcG7 (Cat.No. 551395) followed by Sav-APC (Cat. No. 554067). For analysis, a gate was set to include only viable lymphocytes. In **A**, the histogram shows staining with anti-pig CD16 mAb FcG7. Expression of CD4a and CD8a on CD16⁻ (**B**) and CD16⁺ (**C**) lymphocytes is shown.



Swine Natural Killer Cells *(continued from page 39)*

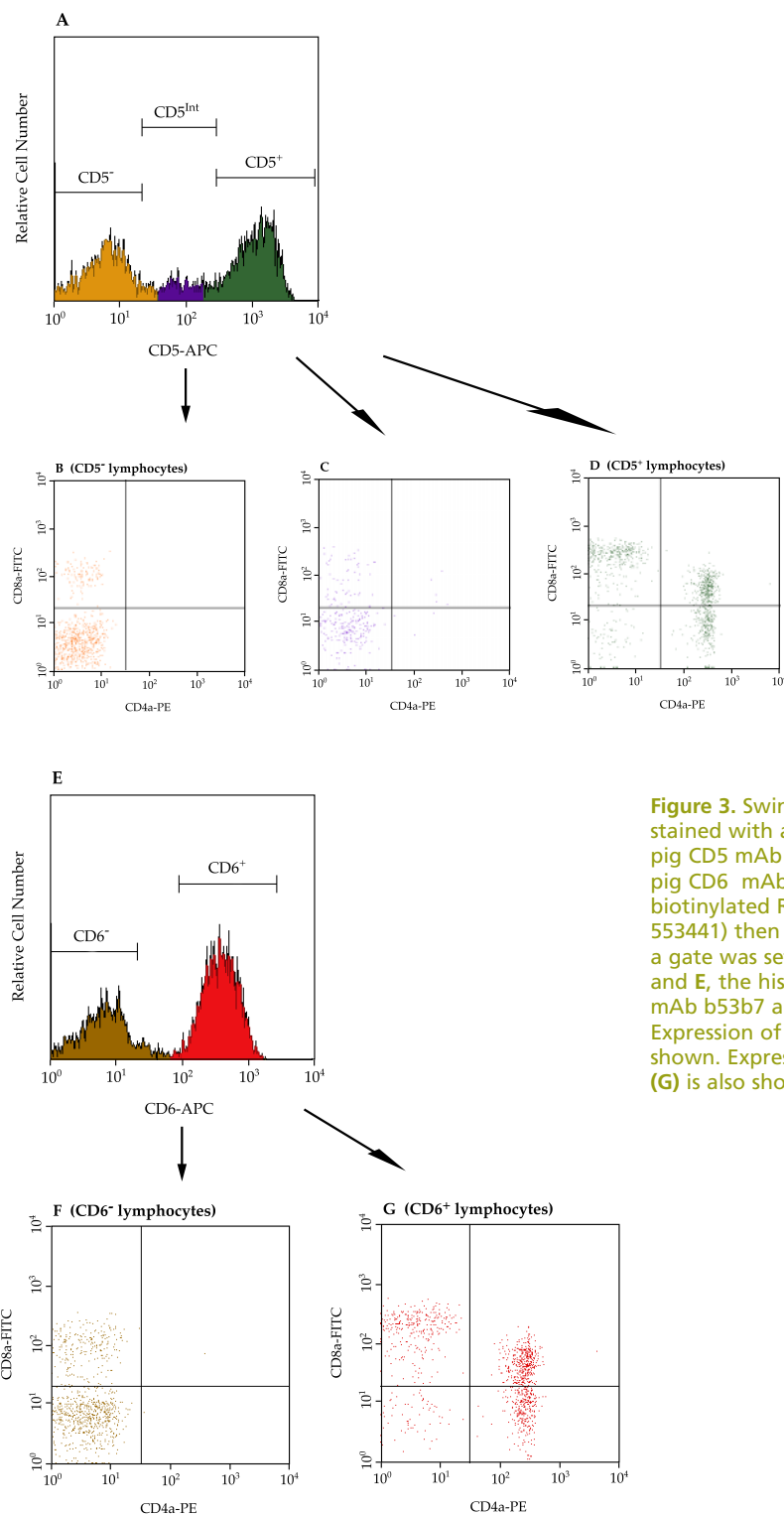


Figure 3. Swine peripheral blood was simultaneously stained with anti-pig CD4 PE, anti-pig CD8, purified anti-pig CD5 mAb b53b7 (Cat. No. 551541) or purified anti-pig CD6 mAb a38b2 (Cat. No. 551507) followed by biotinylated Rat anti-mouse IgG1 mAb A85-1 (Cat. No. 553441) then SAv-APC (Cat. No. 554067). For analysis, a gate was set to include only viable lymphocytes. In **A** and **E**, the histograms show staining with anti-pig CD5 mAb b53b7 and anti-pig CD6 mAb a38b2, respectively. Expression of CD4 and CD8 on CD5⁻ (**B**) and CD5⁺ (**D**) is shown. Expression of CD4 and CD8 on CD6⁻ (**F**) and CD6⁺ (**G**) is also shown.

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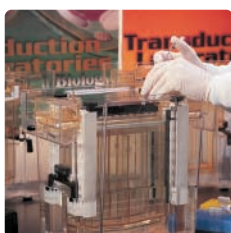
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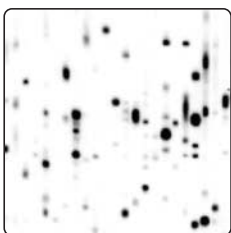


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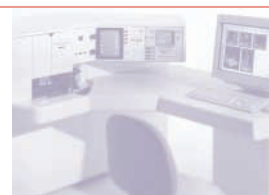
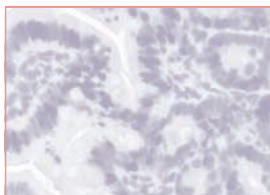
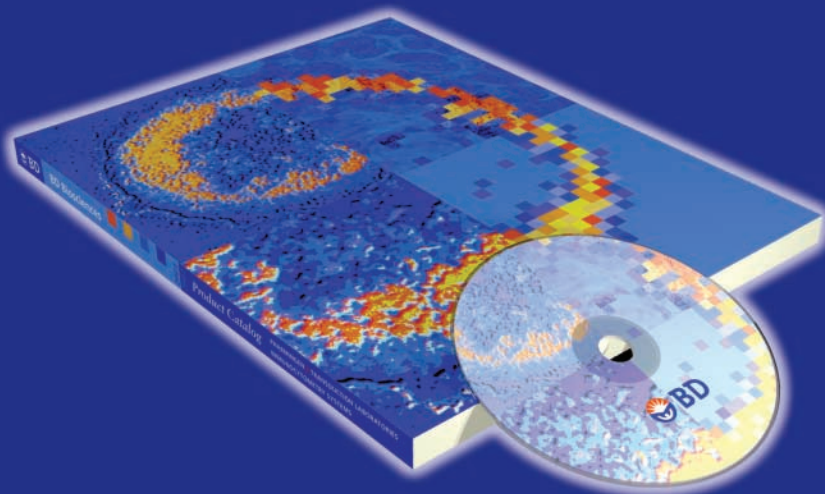
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551456	8108HE	Acinus	Rabbit pAb	Purified	100 ul	
551429	8103HE	AIF (Apoptosis-Inducing Factor)	Rabbit pAb	Purified	100 ul	
550651	80101F	Aquaporin 1	Rabbit pAb	Purified	50 ul	
550650	80101M	Aquaporin 1	Rabbit pAb	Purified	200 ul	
550649	80091F	Aquaporin 2	Rabbit pAb	Purified	50 ul	
550648	80091M	Aquaporin 2	Rabbit pAb	Purified	200 ul	
550647	80081F	Aquaporin 3	Rabbit pAb	Purified	50 ul	
550646	80081M	Aquaporin 3	Rabbit pAb	Purified	200 ul	
612072	A34020-050	ASAP1	19	Purified	50 ug	
612073	A34020-150	ASAP1	19	Purified	150 ug	
551432	8106HE	BACE (β -site APP Cleaving Enzyme)	Rabbit pAb	Purified	100 ul	
612110	B33720-050	BAF47	25	Purified	50 ug	
612111	B33720-150	BAF47	25	Purified	150 ug	
612112	B35220-050	Beclin	20	Purified	50 ug	
612113	B35220-150	Beclin	20	Purified	150 ug	
612130	C20824-050	E-Cadherin	36	FITC	50 ug	
612131	C20824-150	E-Cadherin	36	FITC	150 ug	
551241	80831F	Caspase-7	11-1-56.1	Purified	150 ug	
551240	80831N	Caspase-7	11-1-56.1	Purified	50 ug	
551430	8104HE	Caspase-12	Rabbit pAb	Purified	100 ul	
551443	8107HE	Caspase-13	Rabbit pAb	Purified	100 ul	
550873	80641F	Caspase-14	70A1426	Purified	150 ug	
551526	81096N	Cdk1/Cdk2	AN21.2	Purified	50 ug	
551527	81096F	Cdk1/Cdk2	AN21.2	Purified	150 ug	
612074	C28020-050	Coilin	56	Purified	50 ug	
612075	C28020-150	Coilin	56	Purified	150 ug	
612076	C34120-050	Collybistin	3	Purified	50 ug	
612077	C34120-150	Collybistin	3	Purified	150 ug	
612078	C34520-050	CRP2	1	Purified	50 ug	
612079	C34520-150	CRP2	1	Purified	150 ug	
611911	611911	Daudi Cell Lysate		Lysate	500 ug	
550945	80446E	DRAK2		Purified	200 ul	
612090	D34020-050	β -Dystroglycan	56	Purified	50 ug	
612091	D34020-150	β -Dystroglycan	56	Purified	150 ug	
612114	E35520-050	EPLIN	20	Purified	50 ug	
612115	E35520-150	EPLIN	20	Purified	150 ug	
551527	81106E	Fractin (Cleaved Actin)	Rabbit pAb	Purified	100 ul	
612092	G34320-050	GRASP55	21	Purified	50 ug	
612093	G34320-150	GRASP55	21	Purified	150 ug	
611872	H31020-050	HDJ-2	30	Purified	50 ug	
611873	H31020-150	HDJ-2	30	Purified	150 ug	
611886	H11320-050	Headpin	31	Purified	50 ug	
611887	H11320-150	Headpin	31	Purified	150 ug	
611888	H11620-050	Headpin	49	Purified	50 ug	
611889	H11620-150	Headpin	49	Purified	150 ug	
612066	612066	HepG2+ IL-6 Ctrl Lysate		Lysate	500 ug	
612067	612067	HepG2+IL-6 (15') Lysate		Lysate	500 ug	
612118	H35920-050	Hip1R	44	Purified	50 ug	
612119	H35920-150	Hip1R	44	Purified	150 ug	
611786	611786	Human Carcinoma I Lysate Kit		Lysate	1 Kit	
551818	81171N	IKB α , Phospho-Specific	39A1413	Purified	50 ug	
612120	J36020-050	JAM-1	43	Purified	50 ug	
612121	J36020-150	JAM-1	43	Purified	150 ug	
550959	8076KK	Jurkat Apoptotic Lysate Set I	Set	Lysate	1 mg	
550687	80271M	K ⁺ Channel Kv1.2	Rabbit pAb	Purified	200 ul	
550685	80261M	K ⁺ Channel Kv1.3	Rabbit pAb	Purified	200 ul	
550680	80231F	K ⁺ Channel Kv1.6	Rabbit pAb	Purified	50 ul	
550679	80231M	K ⁺ Channel Kv1.6	Rabbit pAb	Purified	200 ul	

New Cat No.	Cat. No.	Description	Clone	Format	Size	Price(\$)
Cell Biology Reagents (continued)						
550678	80221F	K+ Channel Kv2.1	Rabbit pAb	Purified	50 ul	
550677	80221M	K+ Channel Kv2.1	Rabbit pAb	Purified	200 ul	
550675	80211F	K+ Channel Kv3.1b	Rabbit pAb	Purified	50 ul	
550673	80211M	K+ Channel Kv3.1b	Rabbit pAb	Purified	200 ul	
550655	80121F	K+ Channel SK3	Rabbit pAb	Purified	50 ul	
550654	80121M	K+ Channel SK3	Rabbit pAb	Purified	200 ul	
612094	K34220-050	KIF1A	16	Purified	50 ug	
612096	K35620-050	KIF2	7	Purified	50 ug	
612097	K35620-150	KIF2	7	Purified	150 ug	
612080	M32220-050	MAPKAPK-5	50	Purified	50 ug	
612081	M32220-150	MAPKAPK-5	50	Purified	150 ug	
612082	M35020-050	α -Methylacyl-CoA Racemase	15	Purified	50 ug	
612083	M35020-150	α -Methylacyl-CoA Racemase	15	Purified	150 ug	
612100	N41320-050	Neurotensin Receptor 3	48	Purified	50 ug	
612101	N41320-150	Neurotensin Receptor 3	48	Purified	150 ug	
612098	N34920-050	NSP1	6	Purified	50 ug	
612099	N34920-150	NSP1	6	Purified	150 ug	
550871	80631F	Nucleosome	6E5	Purified	150 ug	
551358	8102HN	PAK4	E440-883	Purified	50 ug	
551528	81114E	PARP (Cleavage Site-Specific)	Rabbit pAb	Purified	200 ul	
612084	P34620-050	Peroxiredoxin V	44	Purified	50 ug	
612085	P34620-150	Peroxiredoxin V	44	Purified	150 ug	
612102	P35820-050	Phosphatase Methylesterase-1	8	Purified	50 ug	
612103	P35820-150	Phosphatase Methylesterase-1	8	Purified	150 ug	
550694	80301F	Purinergic Receptor p2X7	Rabbit pAb	Purified	50 ul	
550693	80301M	Purinergic Receptor p2X7	Rabbit pAb	Purified	200 ul	
551431	8105HE	RAIDD/CRADD	Rabbit pAb	Purified	100 ul	
612086	S34220-050	SCAMP1	22	Purified	50 ug	
612087	S34220-150	SCAMP1	22	Purified	150 ug	
612088	S34420-050	Selenocysteine Lyase	32	Purified	50 ug	
612089	S34420-150	Selenocysteine Lyase	32	Purified	150 ug	
612104	S57320-050	SGT1	29	Purified	50 ug	
612105	S57320-150	SGT1	29	Purified	150 ug	
551821	8119HE	Smac/DIABLO	Rabbit pAb	Serum	100 ul	
511816	8116GN	Syk	4D10	Purified	50 ug	
511817	8116GF	Syk	4D10	Purified	150 ug	
612122	T36120-050	TIP120	48	Purified	50 ug	
612123	T36120-150	TIP120	48	Purified	150 ug	
551357	8101HE	TOAD-64	Rabbit pAb	Serum	100 ul	
551316	80951A	TROP-1	162-21	Purified	0.1 mg	
551317	80961A	TROP-2	162-46	Purified	0.1 mg	
612106	T34120-050	Tubby	40	Purified	50 ug	
612107	T34120-150	Tubby	40	Purified	150 ug	
612126	X37320-050	XPF	26	Purified	50 ug	
612127	X37320-150	XPF	26	Purified	150 ug	
612108	Z34720-050	ZIP Kinase	17	Purified	50 ug	
612109	Z34720-150	ZIP Kinase	17	Purified	150 ug	
612128	Z37520-050	ZPR1	8	Purified	50 ug	
612129	Z37520-150	ZPR1	8	Purified	150 ug	

NEW Products *(continued from page 45)*

New Cat No.	Cat. No.	Description	Clone	Format	Size	Price(\$)
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Cytokine and Chemokine Reagents

Antibodies for Sandwich ELISA

551403	25301D	Rat anti-human sCD14 (ELISA Capture)	55-3	Purified	0.5 mg	
551405	25312D	Mouse anti-human sCD14 (ELISA Detection)	3-C39	Biotin	0.5 mg	
551388	25281D	Mouse anti-human sIL-1RI (ELISA Capture)	hIL-1R-M1	Purified	0.5 mg	
551389	25292D	Mouse anti-human sIL-1RI (ELISA Detection)	hIL-1R-M8	Biotin	0.5 mg	
550823	24411D	Mouse anti-pig IL-1 β (ELISA Capture)	4B2.	Purified	0.5 mg	
550824	24422D	Mouse anti-pig IL-1 β (ELISA Detection)	6E8.10	Biotin	0.5 mg	
550604	23401D	Hamster anti-mouse IL-1 α (ELISA Capture)	ALF-161	Purified	0.5 mg	
550606	23932D	Rabbit anti-mouse IL-1 α (ELISA Detection)	Rabbit Ig	Biotin	0.5 mg	
550605	23411D	Hamster anti-mouse IL-1 β (ELISA Capture)	B122	Purified	0.5 mg	
550623	23942D	Rabbit anti-mouse IL-1 β (ELISA Detection)	Rabbit Ig	Biotin	0.5 mg	
551309	25251D	Rat anti-mouse IFN γ (ELISA Capture)	AN-18	Purified	0.5 mg	
551506	18182D	Rat anti-mouse IFN γ (ELISA Detection)	R4-6A2	Biotin	0.5 mg	

Antibodies for Staining and Flow Cytometric Analysis

551087	25131A	Mouse anti-human C1qRp	R139	Purified	0.1 mg	
551531	25134A	Mouse anti-human C1qRp	R139	PE	0.1 mg	
551509	25135B	Mouse anti-human C1qRp	R139	FITC	0.2 mg	
551454	25351A	Mouse anti-human C1qRp	R3	Purified	0.1 mg	
550900	23892D	Mouse anti-human TNFRI	MABTNFR1-B1	Biotin	0.5 mg	
551074	20072C	Mouse IgG2a isotype control	G155-178	Biotin	0.25 mg	
551359	25271D	Mouse anti-human TNFR Related Protein (LTbR)	hTNFR-RP-M12	Purified	0.5 mg	
551861	25272X	Mouse anti-human TNFR Related Protein (LTbR)	hTNFR-RP-M12	Biotin	100 tests	
551503	25275B	Mouse anti-human TNFR Related Protein (LTbR)	hTNFR-RP-M12	PE	0.2 mg	
551284	25171D	Mouse anti-human GM-CSFR α	hGMCSFR-M1	Purified	0.5 mg	
551412	25172D	Mouse anti-human GM-CSFR α	hGMCSFR-M1	Biotin	0.5 mg	
551373	25175B	Mouse anti-human GM-CSFR α	hGMCSFR-M1	PE	0.2 mg	
551462	25371D	Mouse anti-human IL-6R α	M5	Purified	0.5 mg	
551851	25372X	Mouse anti-human IL-6R α	M5	Biotin	100 tests	
551850	25375X	Mouse anti-human IL-6R α	M5	PE	100 tests	
551894	25701A	Mouse anti-human IL-4R α	hIL4R-M57	Purified	0.1 mg	
551455	25361D	Mouse anti-mouse IL-12R β 1	114	Purified	0.5 mg	
551853	25571A	Rat anti-mouse IL-4R	IL4R-M1	Purified	0.1 mg	
551311	25261D	Rat anti-human TNFRII	hTNFR-M1	Purified	0.5 mg	

Antibodies for Bioassay and Functional Studies (No Azide/Low Endotoxin)

557538	23420C	Hamster anti-mouse IL-1R1	Jama-141	NA/LE	0.25 mg	
551795	25390D	Mouse anti-human IFN- α	7N4-1	NA/LE	0.5 mg	

BD OptEIA™ CL ELISA Kits (Chemiluminescent)

551794	2759KK	Human IL-2 Chemiluminescent Kit		Kit	1 plate	
551501	2755KK	Human IFN- γ Chemiluminescent Kit		Kit	1 plate	
551502	2756KK	Human TNF- α Chemiluminescent Kit		Kit	1 plate	

BD OptEIA™ ELISA Kits (Colorimetric)

550947	2735KK	Human C4a-desArg		Kit	1 plate	
550611	2723KK	Human IL-2 Kit II		Kit	2 plates	
550614	2720KK	Human IL-4 Kit II		Kit	2 plates	
550949	2733KK	Human IL-5 Kit II		Kit	2 plates	
550799	2729KK	Human IL-6 Kit II		Kit	2 plates	
550999	2741KK	Human IL-8 Kit II		Kit	2 plates	

New Cat No.	Cat. No.	Description	Clone	Format	Size	Price(\$)
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Cytokine and Chemokine Reagents

BD OptEIA™ ELISA Kits (Colorimetric) (continued)

550613	2721KK	Human IL-10 Kit		Kit	2 plates	
550801	2731KK	Mouse IL-2 Kit		Kit	2 plates	
550997	2739KK	Mouse IL-5 Kit		Kit	2 plates	
550950	2734KK	Mouse IL-6 Kit		Kit	2 plates	
551423	2746KK	Mouse IL-12 p40 Kit		Kit	2 plates	

BD OptEIA™ Sets (Colorimetric)

550926	2732KI	Human IP-10 Set		Set	Rgts for 20 plates	
551424	2747KI	Human sICAM-1 Set		Set	Rgts for 20 plates	
550995	2737KI	Human TNF-β Set		Set	Rgts for 20 plates	
550996	2738KI	Human TNFRI Set		Set	Rgts for 20 plates	
550948	2736KI	Human TRAIL Set		Set	Rgts for 20 plates	
551492	2750KI	Monkey IFN-γ Set		Set	Rgts for 20 plates	
551494	2752KI	Monkey IL-2 Set		Set	Rgts for 20 plates	
551495	2753KI	Monkey IL-4 Set		Set	Rgts for 20 plates	
551496	2754KI	Monkey IL-6 Set		Set	Rgts for 20 plates	
551493	2751KI	Monkey TNF-α Set		Set	Rgts for 20 plates	
551866	2760KI	Mouse IFN-γ Set (AN-18)		Set	Rgts for 20 plates	
559753	2668KI	Mouse MIP-1β Set		Set	Rgts for 20 plates	
550319	2705KI	Rat IL-6 Set		Set	Rgts for 20 plates	

Human Cell Surface Reagents

551059	35818X	Mouse anti-human β7 integrin	FIB504	Cy-Chrome	100 tests	
551082	35819X	Mouse anti-human β7 integrin	FIB504	APC	100 tests	
551340	38381A	Mouse anti-human Bcl-10	151	Purified	0.1 mg	
550935	38241A	Mouse anti-human μ-Calpain	B27D8	Purified	0.1 mg	
550936	38245X	Mouse anti-human μ-Calpain	B27D8	PE	100 tests	
551312	38371A	Mouse anti-human c-Mpl	BAH-1	Purified	0.1 mg	
551313	38375X	Mouse anti-human c-Mpl	BAH-1	PE	100 tests	
551314	38379X	Mouse anti-human c-Mpl	BAH-1	APC	100 tests	
550796	3010TA	Mouse anti-human CD3	UCHT1	PerCP-Cy5.5	0.1 mg	
550788	3015TA	Mouse anti-human CD4	RPA-T4	PerCP-Cy5.5	0.1 mg	
550791	3032TA	Mouse anti-human CD8	RPA-T8	PerCP-Cy5.5	0.1 mg	
551131	30448X	Mouse anti-human CD11a/LFA-1	HI111	Cy-Chrome	100 tests	
550787	3054TA	Mouse anti-human CD14	M5E2	PerCP-Cy5.5	0.1 mg	
551060	35659X	Mouse anti-human CD18	6.7	APC	100 tests	
550789	3065TA	Mouse anti-human CD19	HIB19	PerCP-Cy5.5	0.1 mg	
551064	30698X	Mouse anti-human CD21	B-ly4	Cy-Chrome	100 tests	
551141	31138X	Mouse anti-human CD42b	HIP1	Cy-Chrome	100 tests	
550988	38271A	Mouse anti-human CD44	515	Purified	0.1 mg	
551063	33508X	Mouse anti-human CD79b	CB3-1	Cy-Chrome	100 tests	
550813	38094X	Mouse anti-human Cw60	UM-4D4	FITC	100 tests	
551137	36008X	Mouse anti-human CDw137	4B4-1	Cy-Chrome	100 tests	
551138	36888X	Mouse anti-human CD161 (NKR-P1A)	DX12	Cy-Chrome	100 tests	
550951	38251A	Mouse anti-human Cytokeratin 14,15,16 and 19	KA4	Purified	0.1 mg	
551264	38304X	Mouse anti-human DC-SIGN	DCN46	FITC	100 tests	
551285	38314A	Mouse anti-human HLA-A2	BB7.2	FITC	0.1 mg	
551127	35749X	Mouse anti-human IL-8 RB	6C6	APC	100 tests	
551056	38291A	Mouse anti-human PRR2	R2.525	Purified	0.1 mg	
551292	38331A	Mouse anti-human TAP2	TAP2.17	Purified	0.1 mg	
550940	08271A	Mouse anti-human TIMP-1	102D1	Purified	0.1 mg	
550795	3381TA	Mouse IgG1, kappa isotype control	MOPC-21	PerCP-Cy5.5	0.1 mg	
550915	38174K	IP30 FITC Set		FITC	set	

NEW Products *(continued from page 47)*

New Cat No.	Cat. No.	Description	Clone	Format	Size	Price(\$)
Ig/2 Step Reagents						
551505	12602A	F(ab') ₂ Rat anti-mouse IgG (multiple adsorption)		Biotin	0.1 mg	
Immunohistochemistry Reagents						
550891	7581KZ	BrdU		Buffer	5 mg	
551249	76101E	Mouse anti-human DC-SIGN	DCN46	Purified	1 ml	
551320	76241E	Mouse anti-mouse follicular dendritic cell	FDC-M1	Purified	1 ml	
551004	75851E	Mouse anti-human MMP-7 (Matrilysin)	ID2	Purified	1 ml	
Cytometric Bead Array Products						
551809	551809	Human Th1/Th2 Cytokine Cytometric Bead Array (CBA) Kit - II (with IL-6)		Kit	50 tests	
551811	551811	Human Inflammation Cytometric Bead Array (CBA) Kit		Kit	50 tests	
551810	2428KC	Human Th1/Th2 Cytokine Standards		lyophilized	1 vial	
Mouse Cell Surface Reagents						
551453	28151A	Hamster anti-mouse CD44H	TM-1	Purified	0.1 mg	
551776	28571D	Rat anti-mouse Dendritic Cells	33D1	Purified	0.5 mg	
551769	06401A	Hamster anti-mouse H-2M3	130	Purified	0.1 mg	
551771	28561C	Mouse anti-mouse DO-11.10 Clonotypic TCR	KJ1-26	Purified	0.25 mg	
551772	28565A	Mouse anti-mouse DO-11.10 Clonotypic TCR	KJ1-26	PE	0.1 mg	
551862	28581A	Rat anti-mouse Pre-B Cell Receptor	SL156	Purified	0.1 mg	
551863	28582A	Rat anti-mouse Pre-B Cell Receptor	SL156	Biotin	0.1 mg	
551864	28591A	Rat anti-mouse Lambda 5	LM34	Purified	0.1 mg	
551865	28592A	Rat anti-mouse Lambda 5	LM34	Biotin	0.1 mg	
551891	28601A	Hamster anti-mouse PD-1	J43	Purified	0.1 mg	
551892	28605A	Hamster anti-mouse PD-1	J43	PE	0.1 mg	
Rat Cell Surface Reagents						
551450	22061A	Mouse anti-rat CD5	HIS47	Purified	0.1 mg	
551449	22054A	Mouse anti-rat CD5	OX-19	FITC	0.1 mg	
551402	22175A	Mouse anti-rat CD45RA	OX-33	PE	0.1 mg	
551451	22181A	Mouse anti-rat CD45RC	OX-22	Purified	0.1 mg	
551452	22751A	Mouse anti-rat CD53	OX-44	Purified	0.1 mg	
551398	22475A	Hamster anti-rat CD62L (L-selectin, LECAM-1)	HRL1	PE	0.1 mg	
551458	22961A	Mouse anti-rat CD63 (ME491)	AD1	Purified	0.1 mg	
551396	22675A	Mouse anti-rat CD86 (B7-2)	24F	PE	0.1 mg	
551401	22215A	Mouse anti-rat CD90 (Thy-1)	OX-7	PE	0.1 mg	
551469	22951A	Mouse anti-rat High Affinity IgE receptor (FcεRI)	BC4	Purified	0.1 mg	
551770	22971A	Mouse anti-rat Mast Cells	AR32AA4	Purified	0.1 mg	
Pig Cell Surface Reagents						
551543	07891A	Mouse anti-pig γ δ T Lymphocytes	MAC320	Purified	0.1 mg	
551541	07871A	Mouse anti-pig CD5	b53b7	Purified	0.1 mg	
551507	07821A	Mouse anti-pig CD6	a38b2	Purified	0.1 mg	
551508	07831A	Mouse anti-pig CD11a (Integrin alpha L chain)	BL2F1	Purified	0.1 mg	
551542	07881A	Rat anti-pig CD44H (Pgp-1, Hermes antigen)	MAC329	Purified	0.1 mg	
551536	07841A	Mouse anti-pig CD45RC	3a56	Purified	0.1 mg	
551544	07901A	Mouse anti-pig CD235a (Glycophorin A)	1AC11	Purified	0.1 mg	
551537	07851A	Mouse anti-pig SLA-DR	1F12	Purified	0.1 mg	
551538	07861A	Mouse anti-pig SLA-DQ	BL4H2	Purified	0.1 mg	

New Cat No.	Cat. No.	Description	Clone	Format	Size	Price(\$)
Molecular Biology Reagents - BD RiboQuant™ Multi-Probe Template Sets						
Mouse Cell Cycle Regulator/Complement						
551490	45751P	mComplement RiboQuant Multi-Probe Template Set			10 reactions	
Mouse Angiogenesis						
551418	45765P	mAngio-1 RiboQuant Multi-Probe Template Set			10 reactions	
Mouse Matrix Metalloproteinase						
551276	45760P	mMMP-1 RiboQuant Multi-Probe Template Set			10 reactions	
550249	45761P	mMMP-2 RiboQuant Multi-Probe Template Set			10 reactions	
Mouse Developmental Gene						
550925	45719P	mWnt-1 RiboQuant Multi-Probe Template Set			10 reactions	
550249	45720P	mWnt-2 RiboQuant Multi-Probe Template Set			10 reactions	
Human Cytokines and Chemokines						
551787	45666P	hCK-8 RiboQuant Multi-Probe Template Set			10 reactions	
551488	45665P	hCK-9 RiboQuant Multi-Probe Template Set			10 reactions	
Human DNA Repair Pathway						
550251	45730P	hDisR RiboQuant Multi-Probe Template Set			10 reactions	
Human Matrix Metalloproteinase						
551274	45760P	hMMP-1 RiboQuant Multi-Probe Template Set			10 reactions	
551275	45761P	hMMP-2 RiboQuant Multi-Probe Template Set			10 reactions	
Human Sulfotransferase						
550793	45735P	hTOX-1b RiboQuant Multi-Probe Template Set			10 reactions	
550794	45736P	hTOX-1 RiboQuant Multi-Probe Template Set			10 reactions	

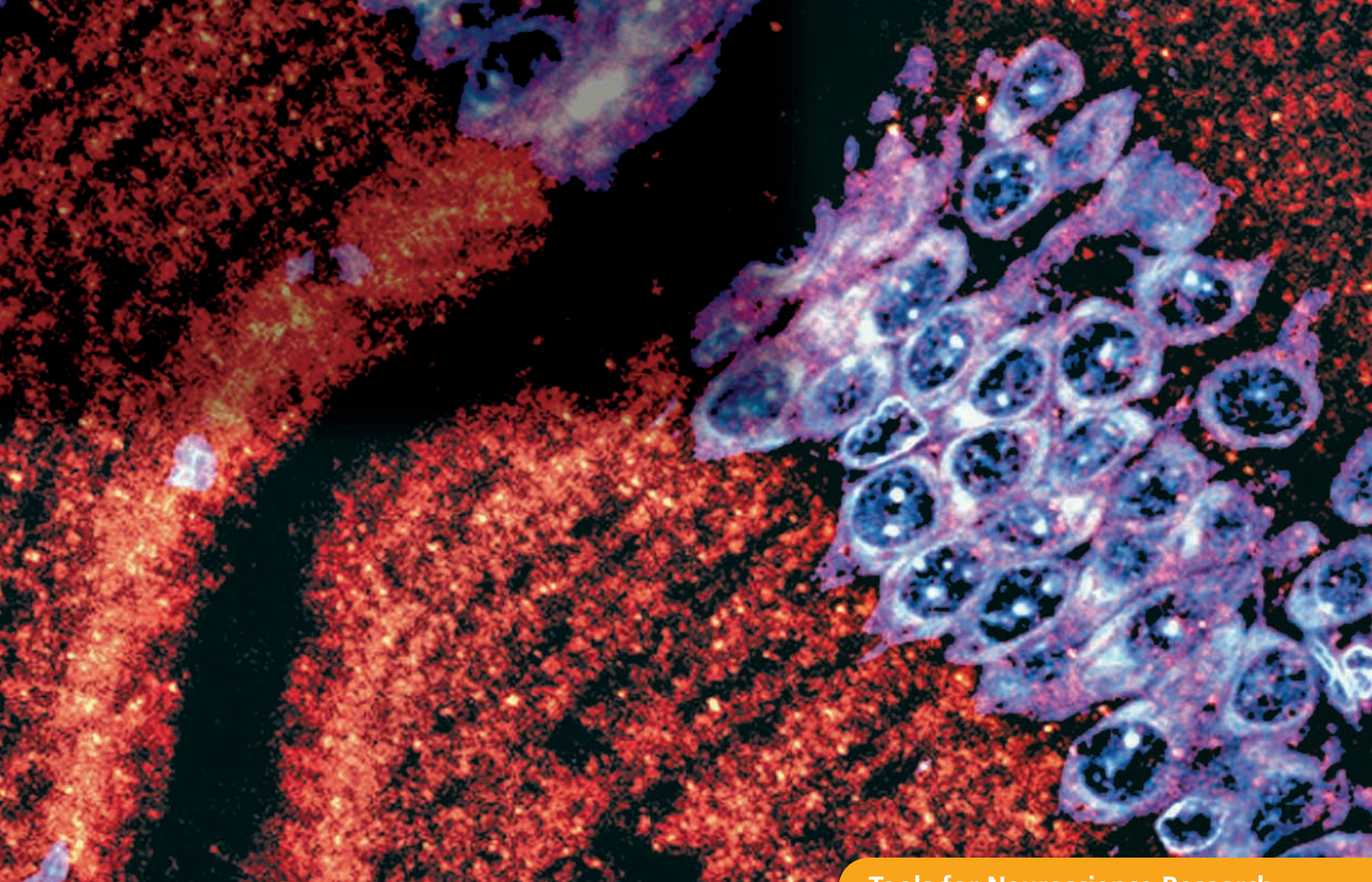
Applicable Patents:

PE and APC: US Patent No. 4,520,110; 4,859,582; 5,055,556. European Patent No. 76,695; and Canadian Patent No. 1,179,942

PerCP: US Patent No. 4,876,190

Cy5.5 and Cy7: US Patent No. 5,268,486; 5,486,616; 5,569,587; 5,569,766; and 5,627,027

APC-Cy7: US Patent No. 5,714,386



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Where We'll Be in Fall 2001

When	What	Where
September 7	University of Wisconsin, Vendor Show	Madison, WI
September 10 -13	Society for Biomolecular Screening (SBS)	Baltimore, MD
September 13	University of California, San Diego, Vendor Show	San Diego, CA
October 4	University of California, Los Angeles, Vendor Show	Los Angeles, CA
October 4 - 5	NIH Research Festival 2001	Bethesda, MD
October 7 - 11	International Society for the Study of Xenobiotics (ISSX)	Munich, Germany
October 12 -14	Great Lakes International Imaging and Flow Cytometry Association Meeting (GLIIFCA)	Wilwaukee, WI
October 12-16	American Society of Bone and Mineral Research (ASBMR)	Phoenix, AZ
October 13 - 17	American Association of Blood Bank (AABB)	San Antonio, TX
October 14 - 17	International Symposium on Laboratory Automation & Robotics (ISLAR)	Boston, MA
October 18	University of California, Irvine, Vendor Show	Irvine, CA
Oct 28 - Nov 1	Chips to Hits	San Diego, CA
November 1	University of Tennessee, Vendor Show	Memphis, TN
November 2 -7	Thymus	San Juan, Puerto Rico
November 7 - 10	Symposium on Non-human Primate Models for AIDS (NHPM)	San Juan, Puerto Rico
November 8 -11	Joint Mtng. International Cytokine Society (ICS) and Society for Leukocyte Biology (SLB)	Maui, Hawaii
November 9 - 11	Biannual Clinical Flow Cytometry Course	Orlando, FL
November 11 - 14	Clinical Applications of Cytometry Meeting (CAC)	Orlando, FL
November 11 - 14	Society for Neuroscience (SFN)	San Diego, CA
November 12 - 14	ADME/Toxicology Meeting	San Diego, CA
November 15 - 17	Autumn Immunology Conference (AIC)	Chicago, IL

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