HotLines

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IN THIS ISSUE:

BD FACSAria™ Flow Cytometer

By Janine Sharpe

Next Generation Flow Cytometry High-Speed Sorter

The BD FACSAria™ cell sorter sets a new standard for high-performance flow cytometry. Based on an entirely new design in instrumentation, the BD FACSAria instrument is the first benchtop sorter that incorporates a fixed-alignment cuvette flow cell. The numerous technological advances embodied in the BD FACSAria cell sorter reduce the cost of owning a high-speed sorter and accelerate research by providing unparalleled, easy-to-use, high performance sorting and analysis.

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BD FACSAria™ Flow Cytometer (continued from cover)

Revolutionary New Optical System

The cuvette flow cell is the heart of the BD FACSAria cell sorter. New technologies incorporated in the instrument design create a truly fixed-alignment optical path. The fixed optical alignment frees the operator from tedious instrument optimization, allowing the focus to center on the science rather than the instrument.

The cuvette flow cell achieves high sensitivity in signal detection with low-powered lasers, allowing air-cooled and solid-state lasers to be utilized in this system. This, in turn, eliminates the need for special power and cooling requirements associated with high-powered lasers used in conventional stream-in-air sorters.

Fiber optics direct and focus the laser light in a precise and constant manner on the sample core stream. Fiber optics steer the three laser beams, 488 nm, 633 nm, and 407 nm, onto the alignment prisms, and then the lasers are focused on the cuvette flow cell *(Figure 1)*. Because the placement of the sample core stream within the cuvette flow cell and the laser alignment are fixed, daily instrument optimization of the cytometer is no longer necessary.

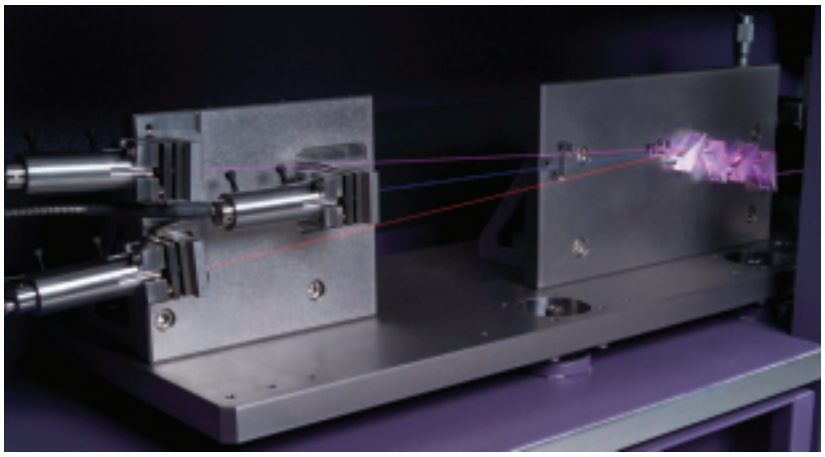

Figure 1. *Excitation optics focusing the 488-nm, 633-nm, and 407-nm laser beams*

Two major advantages of the cuvette flow cell design are improved light excitation and collection optics. Excitation by the BD FACSAria cell sorter differs from a stream-in-air sorter. The sample passes through the laser beams at a lower velocity, allowing longer exposure to laser energy. The stream is accelerated only as it enters the nozzle tip. The drop drive then breaks the stream into droplets to be sorted.

The BD FACSAria instrument is the only high-speed sorter with a gel-coupled cuvette cell that collects at least four times more light than stream-in-air sorters can. This results in improved signal detection sensitivity (<125 Molecules of Equivalent Soluble Fluorescein [MESF])*.

The cuvette flow cell is gel-coupled to the fluorescent objective lens to transmit the greatest amount of light to the collection optics. The collection optics consist of octagon- and trigon-shaped collection devices. The octagon-shaped collection device for the 488-nm laser detects up to seven fluorescent colors plus side scatter. The trigon-shaped collection devices for the 633-nm and 407-nm lasers each collect up to three fluorescent colors. With the addition of FSC, a total of 15 independent signals can be acquired at one time. This entirely new optical system increases the quality and quantity of the information acquired from each sample.

Technological Advances in the Fluidics System

The fluidics system is another key technological advancement embodied in the BD FACSAria system. The fluidics system includes an entirely new means of delivering both the sample and the sheath fluid to the instrument. A new fluidics cart supplies the sheath and cleaning fluids from non-pressurized containers and collects the waste from the instrument *(Figure 3)*. The self-contained fluidics cart supplies air pressure and vacuum eliminating the need for an external source. This feature allows the instrument to operate in any lab or room without the special and costly facility requirements of a traditional high-speed flow cytometer.

**Using Spherotech™ Rainbow RCP-30-5A particles*

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The instrument has a specialized sample-injection chamber that accommodates different tube sizes to provide flexibility in experimental design. Once a tube is placed in the tube holder of the sample-injection chamber, the entire chamber becomes pressurized. This allows even cracked tubes to be sampled and helps sample containment. The tube holder agitates the sample, keeping cells constantly suspended throughout a long sort.

New Era for High-Speed Cell Sorting

With the BD FACSAria cell sorter, high-speed sorting has never been easier to set up and perform. Advances in instrument design give the BD FACSAria system improved sort performance without sacrificing results.

The nozzle tip is keyed into a fixed position at the end of the cuvette ensuring reproducible stream alignment into the waste aspirator every time. Fixed nozzle alignment ensures the stream will return to the same spot after a nozzle tip is removed and reattached.

Figure 2. *ACDU option for plate and slide sorting*

The 70- and 100-micron nozzle tip sizes are designed to accommodate most cell types. The nozzle sizes enable sorting at a variety of pressures and speeds. Two- and four-way sorting are standard features available for a variety of bulk collection devices to accommodate different tube sizes. The optional automated cell deposition unit (ACDU) sorts into BD Multiwell™ plates and onto microscope slides ***(Figure 2)***. The flexibility of the BD FACSAria cell sorter provides the total solution for flow cytometry sorting.

Comprehensive sort monitoring and clog detection are standard integrated software features that make the BD FACSAria cell sorter easy to use. The breakoff monitoring system automatically adjusts the amplitude to maintain the same breakoff value throughout a sort. The clog detection feature signals the instrument to stop the sort and protect the collection tubes if the instrument cannot maintain the stream breakoff.

Advanced digital electronics improve sort performance and provide significant advantages in instrument ease-of-use. The electronics eliminate dead time. This improves the number of signals that can be processed and sorted, thus increasing sort yield by reducing the number of electronic aborts. The electronics simplify compensation of all fluorescent signals with no limitation to inter- and intra-beam compensation.

Faster signal processing enables acquisition rates of up to 70, 000 events per second for ten parameters. The BD FACSAria system can achieve a higher yield in acquisition and sorting than an analog system, which has a minimum dead time of 5.5 microseconds.

Unique Design

The BD FACSAria instrument was engineered to be space-efficient and compact with reduced overall size compared to conventional sorters ***(Figure 3)***. The instrument can be installed and operated in virtually any room without special facility requirements. The instrument can be set up on a typical laboratory bench top or table and requires only a standard electrical outlet.

Figure 3. *BD FACSAria cell sorter with fluidics cart and computer monitor*

Large water-cooled lasers are no longer necessary to achieve high-fluorescent sensitivity and sorting performance. The fluidics system is supported entirely by the self-contained fluidics cart. The BD FACSAria instrument is revolutionary in all aspects of its design and operation.

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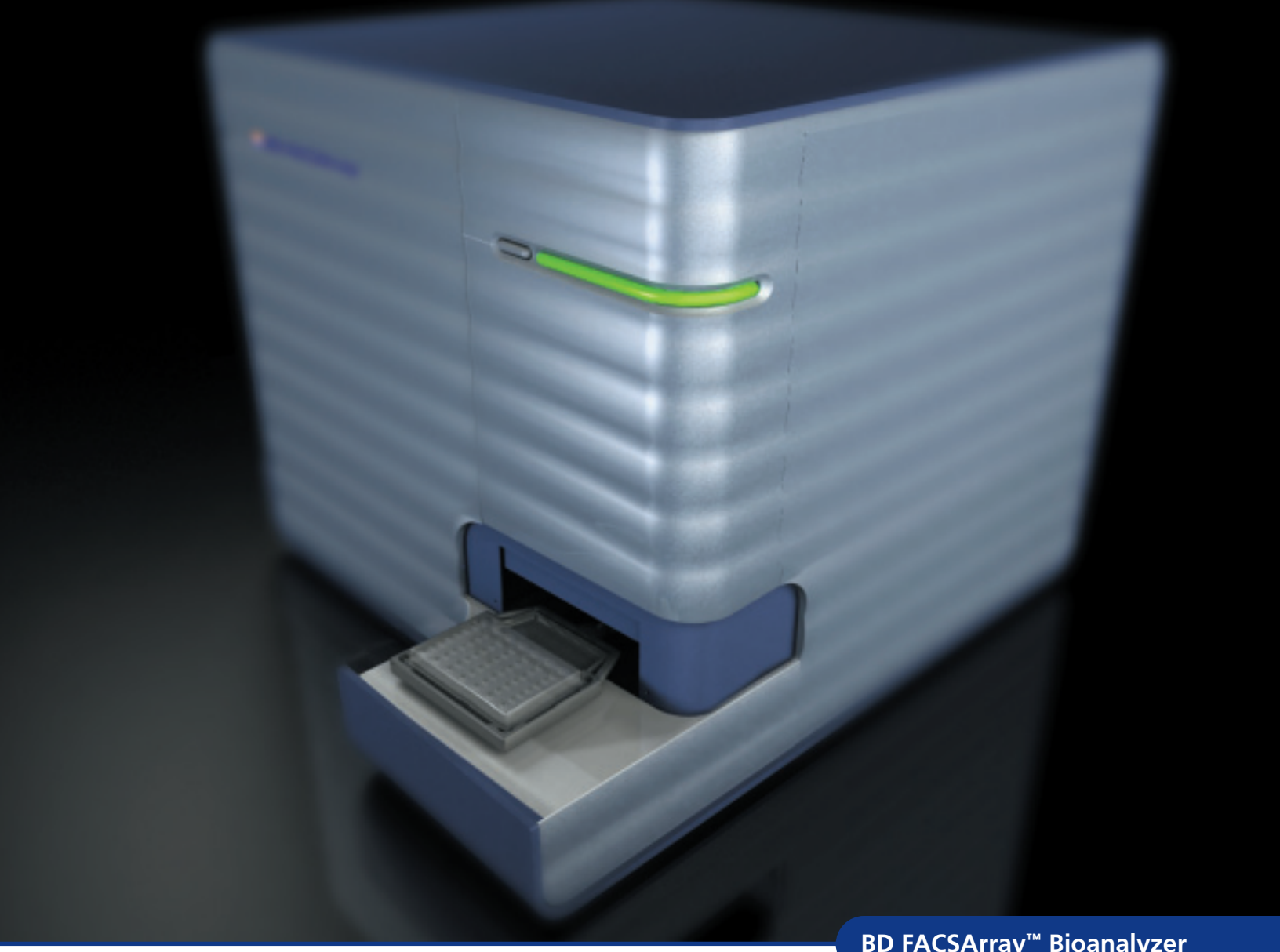

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Capable of collecting six parameters while providing the convenience of walkaway data acquisition with an integrated microtiter well plate sampler.

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SJ-0098-00

The BD™ CBA Human Chemokine Kit - I

By Jing Ping Shih, Ph.D., John King, Jerry Wilson, David Ernst, Ph.D., and Homero Sepulveda, Ph.D.

A powerful tool for assessing levels of multiple chemokines in serum, plasma, or culture supernatant samples

Chemokines are a group of small cytokines, typically 70–80 amino acids in length, that are differentially expressed by a variety of activated cell types. They play critical roles in the development of immune and inflammatory responses by directing leukocyte traffic through the activation and chemotaxis of chemokine receptor-bearing leukocytes. Because of the central roles played by chemokines in directing leukocyte traffic during the generation and expression of inflammatory and immune responses, the analysis of the types and levels of different chemokines that are present in complex biological fluids is of great research interest. To facilitate this research, BD Biosciences has recently developed a flow cytometric bead-based immunoassay capable of simultaneously measuring the levels of five different chemokines from a single serum, plasma, or cell culture supernatant sample, called the BD™ Cytometric Bead Array (BD CBA) Human Chemokine Kit – I.

The BD CBA Human Chemokine Kit – I is capable of specifically and simultaneously measuring human Interleukin-8 (CXCL8/IL-8), RANTES (CCL5/RANTES), Monokine-induced by Interferon-γ (CXCL9/MIG), Monocyte Chemoattractant Protein-1 (CCL2/MCP-1), and Interferon-γ-induced Protein-10 (CXCL10/IP-10). The kit measures individual chemokines by using fluorescently distinct bead populations coated with capture antibodies. The presence of chemokines bound to the antibody-coated beads is detected with fluorescent detection antibodies when analyzed by flow cytometry ***(Figure 1)***. This method provides certain advantages over other common immunoassays such as ELISAs. For instance, the amount of sample used in the simultaneous quantitation of five chemokines in the BD CBA assay is significantly reduced when compared with ELISA analysis that requires individual samples for each chemokine measurement. In addition, the assay employs a single 3-hour incubation thus limiting the number of liquid handling steps and reducing hands-on time for the investigator. The BD CBA Human Chemokine Kit – I provides researchers with a powerful tool to evaluate the chemokine expression profiles in a variety of biological systems.

The BD CBA Human Chemokine Kit – I offers all of the necessary reagents to quantitate five chemokines simultaneously over a wide, 250-fold, dynamic range (standards are tested from 10–2,500 pg/ml). When coupled with the BD™ CBA Software, the investigator is able to rapidly generate standard curves and determine concentrations of unknown samples ***(Figure 2)***. The sensitivity and reproducibility of the assay were determined and are comparable to, if not better than, other quantitative chemokine immunoassays ***(Tables 1 and 2)***. The chemokine spike linearity and recovery in various sample matrices were evaluated ***(Tables 3 and 4)*** with recovery performance varying for each individual chemokine measured. Normal chemokine levels in serum and plasma samples were determined by testing individual serum and plasma samples from ten normal donors ***(Table 5)***.

In an effort to provide a reference between values determined using the BD CBA Human Chemokine Kit – I and other quantitative chemokine immunoassays, the BD CBA Human Chemokine – I Standards were compared with the corresponding National Institute of Biological Standards and Controls/World Health Organization (NIBSC/WHO) Reference Reagents or International Standards. The chemokines evaluated were the WHO 1ST International Standard for Interleukin-8 (89/520), NIBSC Reference Reagent for RANTES (92/520), and the NIBSC Reference Reagent for MCP-1 (92/794). No Reference Reagents were available from the NIBSC/WHO for CXCL9/MIG and CXCL10/IP-10 for use in this comparison. For each chemokine a concentration conversion factor was calculated based on the reported concentration of the BD CBA Human Chemokine – I Standards and the NIBSC/WHO standards. This was determined by using both sets of proteins as standards to quantitate an activated human PBMC cell culture supernatant. The calculation for converting BD CBA results to the corresponding NIBSC/WHO standard result is shown on **page 7**.

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The BD™ CBA Human Chemokine Kit - I *(continued from page 5)*

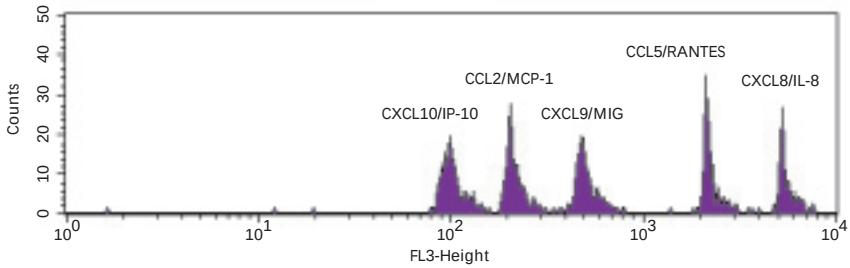

***Figure 1**. Representative fluorescence intensity frequency distributions of the BD CBA Human Chemokine Kit – I Capture Beads. Capture beads were mixed and subsequently analyzed on a BD FACSCalibur™ flow cytometer.*

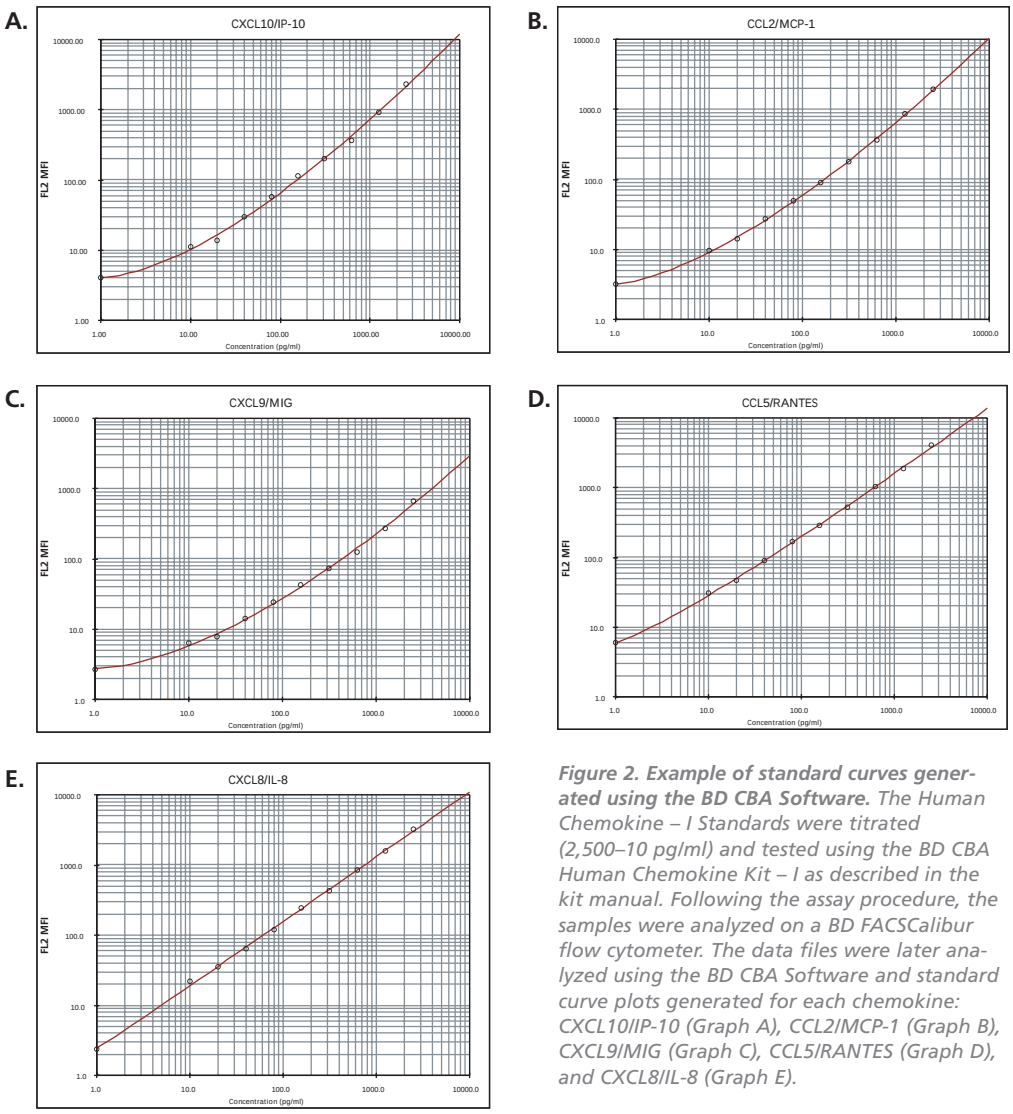

***Figure 2. Example of standard curves generated using the BD CBA Software.** The Human Chemokine – I Standards were titrated (2,500–10 pg/ml) and tested using the BD CBA Human Chemokine Kit – I as described in the kit manual. Following the assay procedure, the samples were analyzed on a BD FACSCalibur flow cytometer. The data files were later analyzed using the BD CBA Software and standard curve plots generated for each chemokine: CXCL10/IP-10 (Graph A), CCL2/MCP-1 (Graph B), CXCL9/MIG (Graph C), CCL5/RANTES (Graph D), and CXCL8/IL-8 (Graph E).*

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NIBSC/WHO Conversions

NIBSC/WHO IL-8 (89/520) equivalent value (pg/ml) = 1.997 × BD CBA IL-8 value (pg/ml)

NIBSC/WHO RANTES (92/520) equivalent value (pg/ml) = 1.026 × BD CBA RANTES value (pg/ml)

NIBSC/WHO MCP-1 (92/794) equivalent value (pg/ml) = 1.053 × BD CBA MCP-1 value (pg/ml)

Non-Human Primate Reactivity

The BD CBA Human Chemokine Kit – I assay has been shown to detect non-human primate CXCL8/IL-8, CCL5/RANTES, and CCL2/MCP-1 produced by the activation of cells from Rhesus and Cynomolgus macaques. Direct quantitation of chemokines in non-human primate samples has not been validated using this kit and results may vary from quantitation of human samples.

The procedure, performance, and the dynamic range of the assay indicate that this will be a robust and useful tool in assessing the chemokine expression profile in a variety of biological samples. For more information, visit our website at **www.bdbiosciences.com** or contact our Technical Services Department at **877.232.8995** (in the US).

Chemokines	Median Fluorescence	Standard Deviation	Assay Sensitivity (pg/ml)
CXCL8/IL-8	2.6	0.2	0.2
CCL5/RANTES	5.5	0.3	1.0
CXCL9/MIG	2.9	0.2	2.5
CCL2/MCP-1	3.3	0.2	2.7
CXCL10/IP-10	4.1	0.3	2.8

Table 1. Sensitivity of the BD CBA Human Chemokine Kit – I. *A group of 20 replicates of the background sample (no chemokines added) were tested using the BD CBA Human Chemokine Kit – I. The samples, along with replicate standard curves, were analyzed using a BD FACSCalibur™ flow cytometer. The resulting data files were reanalyzed using the BD CBA Software and the average median fluorescence intensity (MFI) and standard deviation for each chemokine were calculated for the 20 replicates. By analyzing the background sample results (average MFI + 2 standard deviations) using the standard curves, the theoretical sensitivity for each chemokine was determined.*

Chemokines	Intra-assay Average % CV	Inter-assay Average % CV
CXCL8/IL-8	4.6 %	6.1 %
CCL5/RANTES	7.0 %	10.3 %
CXCL9/MIG	10.4 %	11.9 %
CCL2/MCP-1	6.6 %	8.0 %
CXCL10/IP-10	10.8 %	10.3 %

Table 2. Intra- and Inter-assay reproducibility of the BD CBA Human Chemokine Kit – I. *To determine the intra-assay reproducibility of the BD CBA Human Chemokine Kit – I, ten replicate samples of three different levels of the Human Chemokine – I Standards were tested in a single assay. The average % CV (coefficient of variation) values are depicted in the table (Intra-assay Average % CV). In a similar fashion, to determine the inter-assay reproducibility of the BD CBA Human Chemokine Kit – I, two replicate samples of three different levels of the Human Chemokine – I Standards were tested in four separate experiments. The average % CV values are depicted in the table (Inter-assay Average % CV). For a detailed summary of this data, please refer to the BD CBA Human Chemokine Kit – I Manual.*

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The BD™ CBA Human Chemokine Kit - I *(continued from page 7)*

Chemokines	Cell Culture Media Slope	Pooled Human Sera Slope	Pooled Human Plasma Slope
CXCL8/IL-8	1.00	1.01	1.02
CCL5/RANTES	1.07	*ND	*ND
CXCL9/MIG	1.03	0.97	0.99
CCL2/MCP-1	1.06	0.99	1.06
CXCL10/IP-10	1.00	0.81	0.98

Table 3. Spike linearity results for sera, EDTA-treated plasma, or culture media. *In two experiments, the Human Chemokine – I Standards were spiked into pooled normal sera, pooled normal EDTA-plasma, or culture media and serially diluted. The sample dilutions were tested using the BD CBA Human Chemokine Kit – I and analyzed on a BD FACSCalibur flow cytometer. The slope of the resulting titration curves for each chemokine in each matrix is depicted in the table.*

**ND = Not Determined, the linearity data is not shown for CCL5/RANTES spiked in sera or EDTA-plasma due to the high levels of CCL5/RANTES found in those samples. For a detailed summary of this spike linearity data, please refer to the BD CBA Human Chemokine Kit – I Manual.*

Chemokines	Cell Culture Media % Recovery	Pooled Human Sera % Recovery	Pooled Human Plasma % Recovery
CXCL8/IL-8	99 %	72 %	76 %
CCL5/RANTES	96 %	*ND	*ND
CXCL9/MIG	107 %	70 %	56 %
CCL2/MCP-1	99 %	33 %	33 %
CXCL10/IP-10	99 %	47 %	75 %

Table 4. Spike recovery results for sera, EDTA-treated plasma or culture media. *In two separate experiments, the Human Chemokine – Standards were spiked into various matrices at three different levels. The spiked samples were tested using the BD CBA Human Chemokine Kit – I and the average spike recovery percentage for all three levels spiked into a given matrix are depicted in the table.*

**ND = Not Determined, due to the high levels of CCL5/RANTES in normal serum and EDTA-plasma samples, recovery information could not be generated for this chemokine. For a detailed summary of this spike linearity data, please refer to the BD CBA Human Chemokine Kit – I Manual.*

Sample	Observed CXCL8/IL-8 Range (Average) pg/ml	Observed CCL5/RANTES Range (Average) pg/ml	Observed CXCL9/MIG Range (Average) pg/ml	Observed CCL2/MCP-1 Range (Average) pg/ml	Observed CXCL10/IP-10 Range (Average) pg/ml
Normal Donor Serum (*n* = 10)	< 10 (<10)	10,349–46,704 (21,839)	37–463 (145)	18–152 (77)	232–1,019 (459)
Normal Donor EDTA-Plasma (*n* = 10)	< 10 (<10)	4,382–18,783 (11,388)	48–482 (153)	< 10–57 (34)	202–1,480 (497)

Table 5. Normal serum and EDTA-treated plasma ranges for each chemokine measured using the BD CBA Human Chemokine Kit – I. *Serum and EDTA plasma samples were taken from 10 normal donors and individually tested using the BD CBA Human Chemokine Kit – I. The ranges and average values for each chemokine are depicted in the table.*

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BD™ Cytometric Bead Array Kits Currently Available

DESCRIPTION	CONTENTS	SIZE	CAT. NO.	NEW
Human				
Human Th1/Th2 Cytokine Kit	IL-2, IL-4, IL-5, IL-10, TNF, IFN-γ	50 tests	**550749**	
Human Th1/Th2 Cytokine Kit - II	IL-2, IL-4, IL-6, IL-10, TNF, IFN-γ	50 tests	**551809**	
Human Inflammation Kit	IL-8, IL-1β, IL-6, IL-10, TNF, and IL-12p70	50 tests	**551811**	
Human Active Caspase-3 Kit	Active Caspase-3	100 tests	**552124**	
Mouse				
Mouse Th1/Th2 Cytokine Kit	IL-2, IL-4, IL-5, TNF, IFN-γ	50 tests	**551287**	
Mouse Immunoglobulin Isotyping Kit	Heavy and light chain isotypes of mouse IgG1, IgG2a , IgG2b ,IgG3 , IgA, IgM, IgE	100 tests	**550026**	
Other				
Human Inflammation Cytokine Standards	IL-8, IL-1β, IL-6, IL-10, TNF, IFN-γ, lyophilized	1 vial	**552932**	■
Human Th1/Th2 Cytokine Standards	IL-2, IL-4, IL-5, IL-6, IL-10, TNF, IFN-γ, lyophilized	1 vial	**551810**	
Mouse Th1/Th2 Cytokine Standards	IL-2, IL-4, IL-5, TNF, IFN-γ, lyophilized	1 vial	**552967**	■
BD CBA Software (v1.3)	Mac and PC Compatible CD-Rom and User's guide	1 CD	**550065**	

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

DESCRIPTION	CONTENTS	SIZE	CUSTOM CAT. NO.	NEW
Mouse Inflammation Kit	IL-6, IL-10, MCP-1, IFN-γ, TNF, IL-12p70	50 tests	**552563**	
Human Anaphylatoxin Kit	C4a, C3a, C5a	50 tests	**552690**	■
Human Chemokine Kit - I	IL-8, RANTES, MIG, MCP-1, IP-10	50 tests	**552990**	■
Human Phospho-Stat I	Phosphorylated Stat I		**coming soon**	■

**Orders can be placed directly with BD Biosciences Pharmingen customer service.*

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The BD™ CBA Human Apoptosis Kit

By Anissa Agadir, Ph.D., Jian Gu, Hai Le, Homero Sepulveda, Ph.D., Jerry Wilson, Michael Boyer, Ph.D., and Lisa Stein, Ph.D.

A New Tool for Quantifying Apoptotic Molecules from Cell Lysates.

Apoptosis, or programmed cell death, is essential for tissue homeostasis, and its perturbation can lead to many diseases, including cancer. 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. When apoptosis is induced, procaspases are proteolytically cleaved and reassemble to form active heterotetrameric enzymes (caspases, for cysteine aspartate-specific proteases). The process of their activation is considered to be the key event of apoptosis. Active caspase-3 has emerged as a powerful marker of cells undergoing apoptosis; its presence is considered to be indicative of apoptosis. In living cells the caspases are normally kept inactive by proteins encoded by the Bcl-2 family members, which include pro-apoptotic (example: Bax, Bad) and anti-apoptotic (example: Bcl-2, Bcl-XL) molecules.

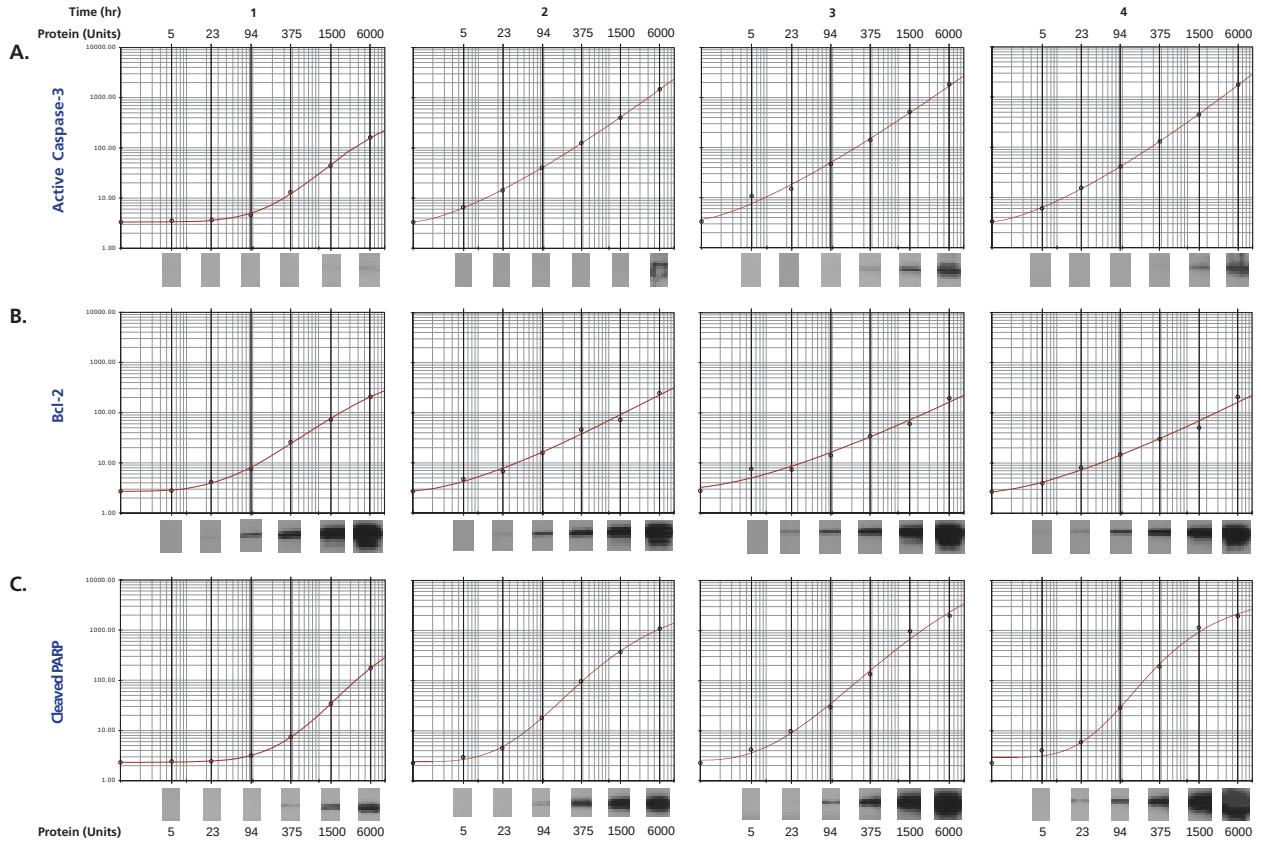

Figure 1. Time course analysis of active Caspase-3, Bcl-2, and cleaved PARP protein levels in Jurkat T Cells. *Cells were treated with 4mM camptothecin for 1hr, 2hr, 3hr, or 4 hr to induce apoptosis. Cells were washed twice with ice-cold PBS, lysed with lysis buffer and analyzed for levels of active caspase-3 (A), Bcl-2 (B), and cleaved PARP (C) using the BD CBA Human Apoptosis Kit. Protein levels are expressed in Units. A unit of active caspase-3, Bcl-2, or cleaved PARP corresponds to the amount of active caspase-3, Bcl-2, or cleaved PARP protein in 0.1µg of total protein from camptothecin-treated Jurkat cell lysate. In addition, parallel samples were tested by western blot (A, B, C). (A): Caspase-3 pAb (poly 1325, Cat. No. 552785), (B) Bcl-2 mAb (clone Bcl-2/100, Cat. No. 556354), and (C) cleavage specific PARP mAb (clone F21-852, Cat. No. 552596) were used for western blot.*

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The cell death regulating activity of the Bcl-2 family members appears to depend on their ability to modulate mitochondria function. The Bcl-2 family members are major regulators of the apoptotic process, whereas caspases are executioners. Another characteristic event of apoptosis is the proteolytic cleavage of poly (ADP-ribose) polymerase (PARP), a nuclear enzyme involved in DNA repair, DNA stability, and transcriptional regulation. Caspases, in particular caspase-3 and -7, cleave the 116-kDa form of PARP into 2 fragments (85 and 24kDa). This cleavage appears to be a marker of the apoptotic process.

The impact of apoptosis on the study of cells has driven the development of new tools to analyze apoptosis. In this study, we developed a novel, sensitive, and reproducible bead-based immunoassay, the BD™ Cytometric Bead Array (BD CBA) (available March/April 2003), in which Active caspase-3, Bcl-2, and cleaved PARP were simultaneously measured from a single cell lysate sample. The assay is capable of measuring these proteins over a two-log dynamic range (the standard is tested from 5–6,000 units for each protein. A unit of active caspase-3, Bcl-2, or cleaved PARP corresponds to the amount of active caspase-3, Bcl-2, or cleaved PARP in 0.1µg of total protein from camptothecin-treated Jurkat cell lysate) using a simple 2 hr assay protocol, providing a significant improvement over conventional western blot assays *(Figure 1)*. *Figure 2* illustrates the 3 protein levels in both control and camptothecin treated U937 and MCF-7 lysates. Active caspase-3 and cleaved PARP are upregulated in camptothecin-treated U937 lysates. In the camptothecin treated MCF-7 lysate, cleaved PARP is upregulated whereas active caspase-3 is not detected; these cells lack expression of caspase-3 due to a mutation in the caspase-3 gene. In both cell lines, Bcl-2 levels remain unchanged after camptothecin treatment. In summary, this highly sensitive assay provides the researcher with a new tool for quantifying apoptotic proteins from cell lysates.

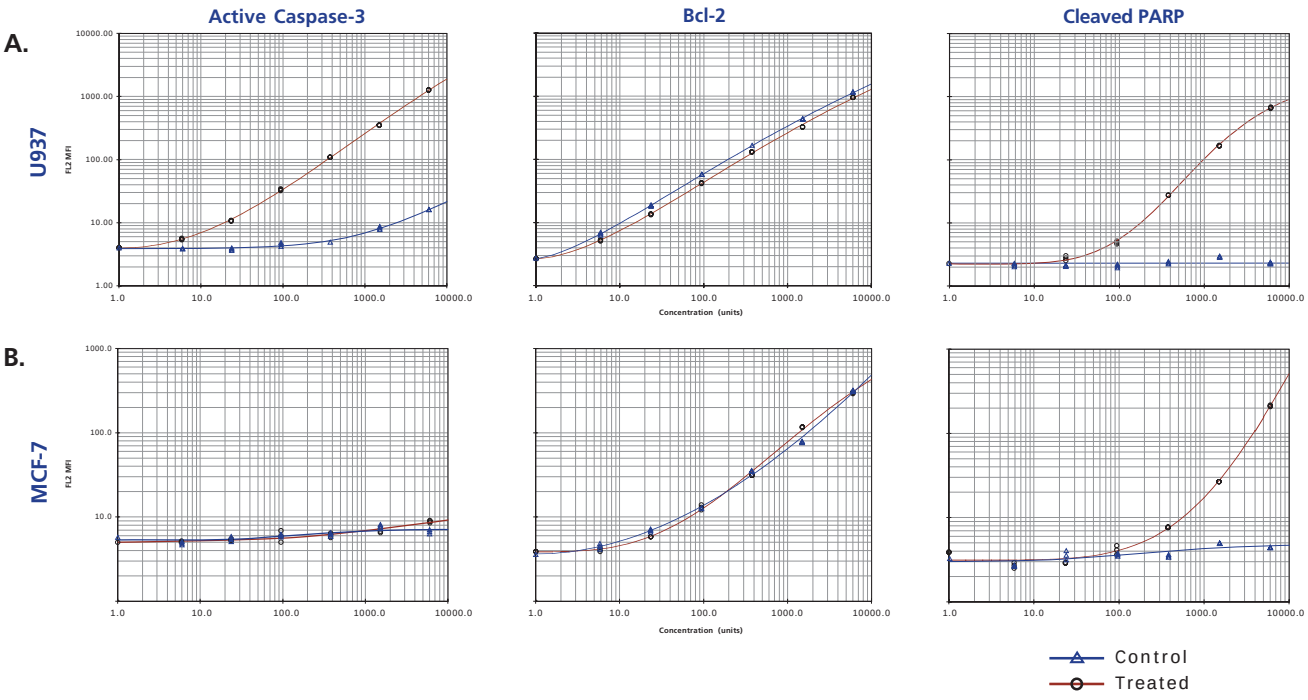

Figure 2. Active Caspase-3, Bcl-2, and cleaved PARP levels in U937 and MCF-7 Cells. *U937 (A) and MCF-7 (B) cells were either untreated (control) or treated with 4mM camptothecin for 4hr to induce apoptosis. Cells were washed twice with ice-cold PBS, lysed with lysis buffer and analyzed for levels of active caspase-3, Bcl-2, and cleaved PARP using a novel, sensitive, and reproducible BD CBA Apoptosis kit. Protein levels are expressed in Units.*

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BD™ DimerX CD1d Reagents

By Bing-Yuan Wei, Ph.D., and Jerry Wilson

A new tool for the study of mouse Natural Killer T cells

BD Biosciences Pharmingen is proud to announce the release of the new BD™ DimerX I: Recombinant Soluble Dimeric Mouse CD1d:Ig fusion protein. The CD1d:Ig fusion protein consists of the extracellular major histocompatibility complex (MHC) class I-like domains of the mouse CD1d molecule fused with the VH regions of mouse IgG_1 ***(Figure 1)***. Recombinant CD1d molecules, like the DimerX fusion protein, are useful for studying Natural Killer T (NKT)-cell function by immunofluorescent staining and flow cytometric analysis of antigen-specific NKT cells.[1,2,3,4]

The mouse CD1D1 gene encodes a non-polymorphic cell-surface protein that plays a role in antigen presentation to CD1d-restricted NKT cells.[1,2,5] Like the MHC class I molecules, CD1d associates non-covalently with β_2 Microglobulin (β_2M) and is capable of binding and presenting lipid antigens.[1] (The mouse CD1d:Ig molecule is co-expressed with mouse β_2M). While the natural ligand for CD1d is presently unknown, it is well documented that CD1d can bind and present the glycolipid, α-galactosyl ceramide (α-gal cer*), a glycosphingolipid from the marine sponge.[1,3] Antigenic glycolipids, such as α-gal cer, associated with the CD1d molecule are presented and specifically recognized by NKT cells expressing a highly conserved TCR, Vα14Jα281, paired with diverse β chains such as Vβ8.2 and Vβ7.[1,2,3] As the BD DimerX molecule can be loaded with a ligand capable of binding in the ligand-binding groove of the CD1d molecule. Therefore, it may be a valuable research tool for determining the natural ligand for Mouse CD1d.

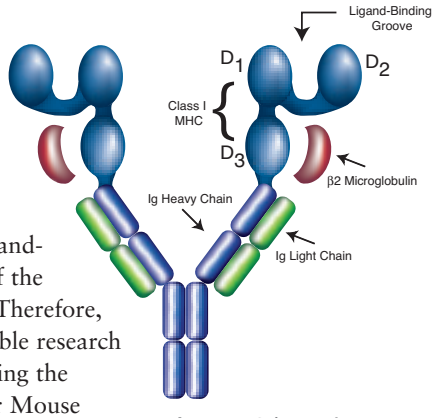

Figure 1. Schematic representation of the CD1d:Ig dimeric protein.

References:

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BD™ DimerX Peptide Presentation Reagents

DESCRIPTION	ISOTYPE	APPS	FORMAT	SIZE	CAT. NO.	PRICE
Human						
Human DimerX I: Recombinant Soluble Dimeric CD1d:Ig Fusion Protein	Mouse IgG_1, λ	FCM	Purified	0.1 mg	**inquire**	inquire
Human DimerX I: Recombinant Soluble Dimeric HLA-A2:Ig Fusion Protein	Mouse IgG_1, λ	FCM	Purified	0.05 mg	**551263**	$495
Mouse						
Mouse DimerX I: Recombinant Soluble Dimeric CD1d:Ig Fusion Protein	Mouse IgG_1, λ	FCM	Purified	0.25 mg	**557599**	$550
Mouse DimerX I: Recombinant Soluble Dimeric H-2D^b:Ig Fusion Protein	Mouse IgG_1, λ	FCM	Purified	0.25 mg	**551323**	$395
Mouse DimerX I: Recombinant Soluble Dimeric H-2K^b:Ig Fusion Protein	Mouse IgG_1, λ	FCM	Purified	0.25 mg	**550750**	$395
Mouse DimerX I: Recombinant Soluble Dimeric H-2K^b:Ig Fusion Protein	Mouse IgG_1, λ	FCM	PE	0.1 mg	**552944**	$495
Mouse DimerX I: Recombinant Soluble Dimeric H-2L^d:Ig Fusion Protein	Mouse IgG_1, λ	FCM	Purified	0.25 mg	**550751**	$395
Other						
Recombinant human β_2 microglobulin		FCM	Purified	0.1 mg	**551089**	$75

**The rights to α-gal cer are owned by Kirin Brewery. The α-gal cer molecule and its derivatives are covered by US Patent No. 5,936,076.*

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The Tumor Necrosis Factor & Lymphotoxin Ligand/Receptor Systems

By Efthalia Chronopoulou, Ph.D., Jeanne Elia, and David N. Ernst, Ph.D.

Tumor Necrosis Factor (aka, TNF, TNF-α) and lymphotoxin (LT) are structurally homologous members of the TNF superfamily of ligands.[1-4] These cytokines play many different roles in the generation and regulation of inflammatory and immune responses.

TNF is produced by activated cell types including monocytes, macrophages, Kupffer's cells, astrocytes, granulocytes, mast cells, T and B lymphocytes, NK cells, keratinocytes, fibroblasts, adipocytes, and certain tumor cells. Leukocytes are induced *in vitro* and *in vivo* by different stimuli including antigens, bacteria, viruses, parasites, immune complexes, complement fragments (C5a), mitogens (lipopolysaccharide), and cytokines (IL-1, -2, -3, -2, -15, colony stimulating factors and interferons) to produce TNF. Various inhibitors of prostaglandin synthesis and phosphodiesterase, cyclosporin A and cytokines (IL-4, -6, -10, -11, -3, and TGF-β) are known to suppress TNF expression. Activated human cells initially express TNF as a Type II transmembrane protein subunit (~26 kD, 233 amino acids). Transmembrane TNF subunits associate to form homotrimeric complexes. Membrane TNF can be cleaved by a membrane-bound, matrix metalloproteinase called TNF-α converting enzyme (TACE, aka, CD156b). In this way, the extracellular region of transmembrane TNF sheds as a soluble, biologically active homotrimer (~50 kD). Under denaturing conditions, the mature, 157-amino-acid-long subunits exhibit a molecular mass of ~17 kDa.

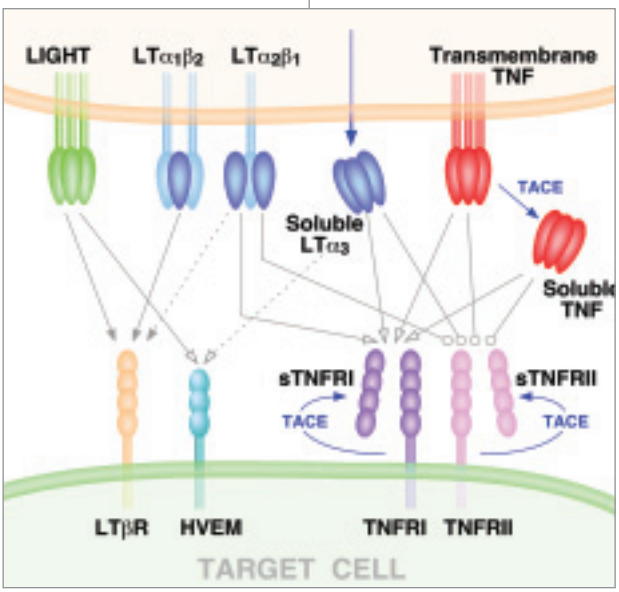

Figure 1. Schematic representation of the TNF/LT cytokine system. *Depicted are the soluble and transmembrane homotrimeric and/or heterotrimeric forms of TNF and LT ligands, respectively. Arrows point to their respective receptors. Solid arrows indicate high affinity while dashed lines indicate low affinity ligand-receptor interactions. HSVγD (the envelope glycoprotein γD of Herpes Simplex Virus) binds with high affinity to HVEM but that interaction is not indicated in the present diagram. LIGHT is a TNF family member that signals via the LTβR and HVEM receptors. Receptors are represented in the diagram as monomers for simplicity.*

Lymphotoxins (LT) are related members of the TNF Superfamily that are expressed by activated lymphocytes (eg, Th1 cells, naïve and memory T cells, B cells, NK cells, Peyer's patch progenitor cells), as secreted cytotoxins or cell surface proteins. Exposure of naïve T cells to Th1-associated cytokines such as IFN-γ and IL-12 does not affect their LTαβ expression while exposure to IL-4 leads to downregulation of membrane lymphotoxin.[5] Secreted human lymphotoxin exists as a soluble homotrimer aka, LTα3 or just LTα, formerly TNF-β that displays a similar spectrum of biological activities (albeit often weaker) as TNF. Lymphotoxin β (LTβ) is a transmembrane protein that can associate with LTα subunits to form surface LT complexes that exist as either LTα1β2 or less-prevalent LTα2β1 heterotrimers. LTα1β2 signals through the LTβ receptor (LTβR, aka, TNFRrp) and is involved in the development and organization of secondary lymphoid tissue and the generation and regulation of immune responses.[4-6] The LTβR is a type I transmembrane glycoprotein (61 kD, 435 amino acids). LTβRs are expressed by stromal cells. Other cells expressing LTβRs include normal bronchial airway epithelial cells, dermal fibroblasts, as well as a variety of adherent cell lines such as HeLa, U937, HT-29, etc. LTβRs also strongly bind the membrane form of another transmembrane TNF family member ligand, LIGHT and can bind LTα2β1 weakly. However the functional significance of LTβR-LTα2β1 interaction has not been clarified yet.

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The TNF & Lymphotoxin Ligand/Receptor Systems (continued from page 13)

In addition, it is not clear whether the LTα2β1 heterotrimer is significantly expressed *in vivo*.

Homotrimeric forms of membrane TNF and soluble LTα and TNF exert their biological activities by binding, crosslinking and signaling through Type I (~55-60 kDa, 426 amino acids) and Type II (~75-80 kDa, 439 amino acids) TNF Receptors (aka, TNFRI/CD120a and TNFRII/CD120b, respectively). Monomeric forms of TNF and LTα are not biologically active. The TNFRs are type II transmembrane glycoproteins whose extracellular regions are comprised of four, well-conserved cysteine-rich domains, but whose intracellular domains are distinctly different. The TNFRI is constitutively expressed by most cell types whereas the TNFRII is expressed primarily by endothelial cells and cells of hematopoietic origin. Transmembrane TNF effects cell signaling (eg, cell death or costimulation) by direct cell-to-cell (juxtacrine) contact. The transmembrane form of TNF may bind preferentially to the TNFRII. Most proinflammatory activities of TNF appear to be mediated through the TNFRI. There is evidence that TNFRII can mediate cytotoxicity and costimulate thymocyte and T cell proliferation.

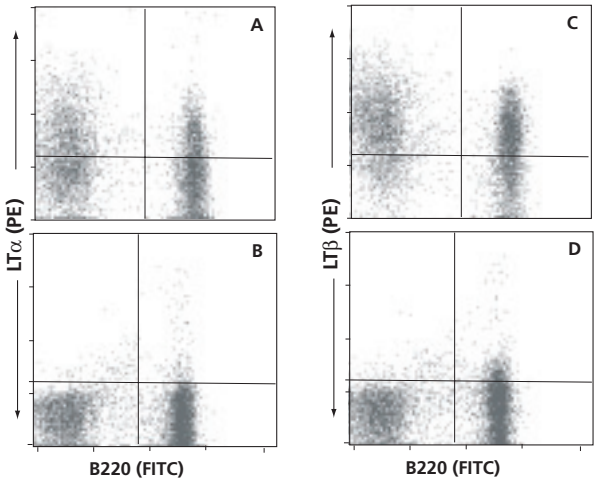

Figure 2. Expression of cell surface LTα2β1 and LTα1β2 by C57BL/6 splenocytes. *C57BL/6 splenocytes were red blood cell-lysed using an ammonium chloride lysing buffer and incubated overnight with (Panels A and C) or without PMA (Panels B and D, 50 ng/ml). Following incubation the cells were harvested and Fc-receptor blocked with mouse Fc Block™ (Cat. No. 553141). The cells were subsequently stained for LTα and LTβ expression with purified AF.B3 (0.25 µg/10⁶ cells, Cat. No. 552937) and BB.F6.F6.BF2 (0.06 µg/10⁶ cells, Cat. No. 552938) respectively, followed by biotinylated goat anti-hamster IgG (Cat. No. 554010), streptavidin-phycoerythrin (0.015 µg, Cat. No. 554061) and FITC-B220 (Cat. No. 553087). Two-color dot plots showing the correlated expression patterns of mouse LTα or LTβ and B220 were derived from gated events with the forward- and side-light scatter characteristics of viable lymphocytes.*

The TNF and LTα ligands also bind to soluble forms of TNFR (sTNFRI and sTNFRII). The sTNFRs represent the extracellular domains of the transmembrane TNF receptors. sTNFRs are naturally shed by activated cells through enzymatic cleavage[7-9] and can be found at elevated levels in the plasma, urine, synovial fluids, and cerebral spinal fluids of diseased individuals or in supernatants from stimulatory cell cultures. The loss of cell surface receptors can lead to a transiently decreased cellular responsiveness to TNF and LTα3. Depending on their relative concentrations, sTNFR can either enhance (eg, TNF stabilization) or inhibit (eg, block TNF binding) TNF's biological activities. It appears that sTNFRs may play a primary role in the clearance of TNF and LTα3 from the blood.

TNF is a multifunctional cytokine that can regulate the growth, proliferation, differentiation, and viability of activated leukocytes and other cell types. TNF can induce upregulated levels of MHC class I and class II molecules by various cell types as well as the increased expression of adhesion molecules by endothelial cells that are relevant to inflammatory and immune responses. TNF can also trigger the cellular release of other cytokines, chemokines or inflammatory mediators, and display antiviral and antimicrobial effects. TNF is selectively cytotoxic for some activated normal cells types (eg, $CD8^+$ T cells) and for many transformed cell lines. Although TNF serves as a primary mediator in protective immune responses against microbial and viral pathogens, it can also drive pathophysiologic responses including septic shock, and autoimmune diseases. Recombinant sTNFR and anti-TNF antibodies have been approved for treating human diseases such as rheumatoid arthritis and inflammatory bowel disease.

Selective gene disruption experiments of LTα and LTβ indicate that lymphotoxin plays a critical role in the embryonic development and organization of secondary lymphoid organ microarchitecture.[10-11] The secondary lymphoid organs are sites where antigens entering the skin or a mucosal surface are trapped and concentrated.

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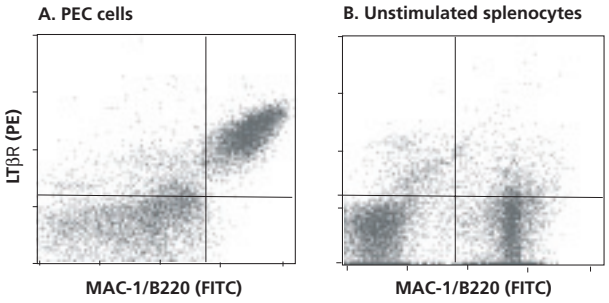

Figure 3. Expression of cell surface LTβR by Peritoneal Exudate Cells (PEC). *Three-day thioglycollate-elicited PEC cells (Panel A) or unstimulated RBC-lysed splenocytes (Panel B) from C57BL/6 mice were washed and Fc-receptor blocked with mouse Fc Block™ (Cat. No. 553141). The cells were subsequently stained with purified AF.H6 (0.06 µg/10^6 cells, Cat. No. 552940) followed by biotinylated goat anti-hamster IgG (Cat. No. 554010), streptavidin-phycoerythrin (0.015 µg, Cat. No. 554061) and either FITC-MAC-1 (Cat. No. 553310) or FITC-B220 (Cat. No. 553087). Two-color dot plots showing the correlated expression patterns of mouse LTβR and MAC-1 (Panel A) or FITC-B220 (Panel B) were derived from gated events with the forward and side light–scatter characteristics of viable mononuclear cells.*

They are also sites where lymphocytes, antigen presenting and transporting cells, or other regulatory cells are located. To optimize the cellular interactions and increase the circulation of naïve T cells where antigens are trapped, the secondary lymphoid tissues are anatomically organized into different compartments known as B and T cell zones. Disturbance of the secondary lymphoid organ microarchitecture reduces the efficiency of immune responses. Membrane lymphotoxin is thought to support the maintenance of this secondary lymphoid organ microarchitecture throughout life. Lymphotoxins can be involved in the initiation of inflammation by inducing adhesion molecules and producing chemokines in response to invading pathogens. Lymphotoxins can induce *in vitro* apotosis in a variety of tumor cell lines indicating that they may contribute in defense against tumors.

BD Biosciences Pharmingen offers a vast number of reagents for studying TNF- and LT- ligand/ receptor biology. For a complete product listing please refer to *Table 1*.

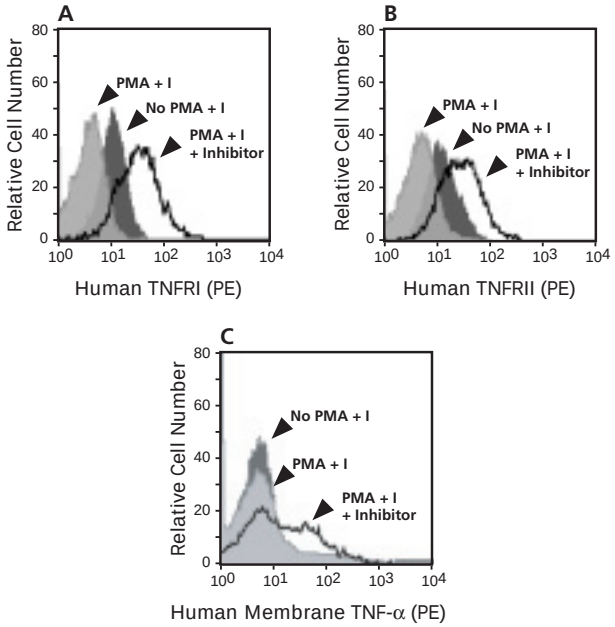

Figure 4. TACE (Tumor Necrosis Factor-α Converting Enzyme) inhibitors block activation induced shedding of TNFRs and membrane TNF. *Human PBMCs isolated by density gradient centrifugation (Ficoll-Paque™) were stimulated with plate–bound anti-human CD3 antibody (10 µg/ml, Cat. No. 555336) and soluble anti-CD28 antibody (2 µg/ml, Cat. No. 555725) in the presence of human IL-2 (10 ng/ml, Cat. No. 554603) and IL-4 (40 µg/ml, Cat. No. 554605) for 2 days. The cells were subsequently washed and expanded in IL-2 and IL-4 for 3 days. Following expansion, the cells were washed and stimulated for 2 hrs with PMA (5 ng/ml) and ionomycin (500 ng/ml) with or without 25 µM of metalloprotease inhibitors (TAPI) or were used without further stimulation. Following incubation, the cells were harvested and their surface expression of human TNFRI and TNFRII were detected by immunofluorescent staining and flow cytometric analysis using biotinylated anti-human TNFRI (clone MABTNFR1-B1, Cat. No. 550900, Panel A) and purified anti-human TNFRII (clone hTNFR1-M1, Cat. No. 551311, Panel B), respectively. The anti-human TNFRI and anti-human TNFRII antibodies were subsequently detected by PE-streptavidin (Cat. No. 554061, Panel A) and biotinylated F(ab')$_2$ goat anti-rat IgG (Jackson ImmunoResearch, Cat. No. 112-066-062, Panel B) followed by PE-streptavidin, respectively. Expression of membrane TNF was detected using the PE-labeled anti-human TNF antibody (clone MAb11, Cat. No. 559321, Panel C). Histograms were derived from gated events with the forward and side light scatter characteristics of viable lymphocytes.*

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The TNF & Lymphotoxin Ligand/Receptor Systems (continued from page 15)

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Table 1. *Please consult the 2003 BD Biosciences Product Catalog and the BD Biosciences Online Catalog at* ***www.bdbiosciences.com/e_catalog*** *for a more extensive listing of TNF Ligand and Receptor Superfamily Reagents. These reagents are designed for studies at the MRNA, protein, and cellular levels.*

DESCRIPTION	SPECIES	CLONE	APPS	FORMAT	CAT. NO.	NEW
TNF-α	Ms	G281-2626	ELISA, WB	Purified	**551225**	
	Ms		FA	NA/LE	**554640**	
	Ms	MP6-XT22	Block, IC/FCM, WB	Purified	**554416**	
	Ms		ICC	Purified	**559064**	
	Ms		IC/FCM	FITC	**554418**	
	Ms		IC/FCM	PE	**554419**	
	Ms		IC/FCM	APC	**554420**	
	Ms	MP6-XT3	FA, Neu	NA/LE	**554414**	
	Ms		ELISA	Biotin	**554415**	
	Ms	Polyclonal	ELISA	Biotin	**557432**	
	Ms, Rat	TN3-19.12	FA	NA/LE	**557532**	
	Ms, Rat		ELISA	Purified	**557516**	
	Ms, Rat		IC/FCM	PE	**559503**	
TNF-α Receptor I (p55)	Ms	55R-170	FA	NA/LE	**557535**	
	Ms	55R-286	FCM, IP/WB	Purified	**559915**	
	Ms	55R-593	FA	NA/LE	**557536**	
TNF-α Receptor II (p75)	Ms	TR75-32	FA	NA/LE	**557533**	
	Ms	TR75-54	FA	NA/LE	**557534**	
	Ms	TR75-89	FCM, IP/WB	Purified	**559916**	
	Ms		FCM	PE	**550086**	
Recombinant TNF-α	Ms		Block, ELISA, FA, IC/FCM	Purified	**554589**	

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DESCRIPTION	SPECIES	CLONE	APPS	FORMAT	CAT. NO.	NEW
TNF-α	Hu	MAb1	ELISA	Purified	**551220**	
	Hu		FA	NA/LE	**554508**	
	Hu	MAb11	Block, IC/FCM	Purified	**554510**	
	Hu		ICC	Purified	**559071**	
	Hu		ELISA	Biotin	**554511**	
	Hu		IC/FCM	FITC	**554512**	
	Hu		IC/FCM	PE	**554513**	
	Hu		IC/FCM	PE	**559321**	
	Hu		IC/FCM	APC	**554514**	
	Hu	MABTNF-A5	FA	NA/LE	**552467**	■
	Bab, Cyno, Rhe	MabII	IC/FCM	Purified	**558882**	
	Bab, Cyno, Rhe		IC/FCM	PE	**557068**	
	Rb	23H1.1	ELISA	Biotin	**552470**	
	Rb		FA	NA/LE	**553629**	■
	Rb		ELISA	Purified	**551214**	
	Rb		ELISA	Biotin	**551213**	
	Rat, Ms	polyclonal	ELISA	Biotin	**557432**	
	Rat, Ms	TN3-19.12	ELISA	Purified	**557516**	
	Rat, Ms		FA	NA/LE	**557532**	
	Rat, Ms		IC/FCM	PE	**559503**	
TNF-α Receptor Type I	Hu	MABTNFR1-B1	FCM	Purified	**550514**	
	Hu		ELISA	Biotin	**552536**	
	Hu		FCM	Biotin	**550900**	
	Hu	MABTNFR1-A1	ELISA	Purified	**552535**	
TNF-α Receptor Type II	Hu	hTNFR-M1	FCM	Purified	**551311**	
	Hu		FCM	Biotin	**552417**	
	Hu		ELISA	Biotin	**552477**	
	Hu		FCM	PE	**552418**	
	Hu		FCM	APC	**552419**	
Recombinant TNF-α	Hu		ELISA, FA	Purified	**554618**	
Recombinant sTNF Receptor I	Hu		ELISA	Purified	**552537**	
Recombinant TNFRII (p75)	Hu		ELISA	Purified	**552499**	
Recombinant soluble TNFR60:FC	Hu		FA	Purified	**557022**	
Recombinant TNF-α	Rat		ELISA, FA	Purified	**555109**	
Lymphotoxin-α	Ms	AF.B3	FCM	Purified	**552937**	■
Lymphotoxin-β	Ms	BB.F6.F6.BF2	FCM	Purified	**552938**	■
Lymphotoxin β receptor	Ms	AF.H6	FCM	Purified	**552940**	■
	Ms	AC.H6	FCM	Purified	**552939**	■
Lymphotoxin-α	Hu	359-238-8	ELISA	Purified	**551222**	
	Hu	359-81-11	FA	NA/LE	**554553**	
	Hu		Block, IC/FCM	Purified	**554554**	
	Hu		ELISA	Biotin	**554555**	
	Hu		IC/FCM	PE	**554556**	
LTα1β2	Hu	AG9.BD6	FCM	Purified	**552873**	
LTα2β1	Hu	BF7.AE2	FCM	Purified	**inquire**	■
Lymphotoxin-β	Hu	B9.C9	FCM	Purified	**552874**	■

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The TNF & Lymphotoxin Ligand/Receptor Systems (continued from page 17)

Table 1. *(continued)*

DESCRIPTION	SPECIES	CLONE	APPS	FORMAT	CAT. NO.	NEW
Lymphotoxin β receptor	Hu	hTNFR-RP-M12	FCM	Purified	551359	
	Hu		FCM	Biotin	551861	
	Hu		FCM	PE	551503	
	Hu	BCG6.AF5	FCM	Purified	552875	■
Recombinant LT-α	Hu		ELISA, FA	Purified	554619	

DESCRIPTION	SPECIES	ASSAY RANGE	CAT. NO.	NEW
BD OptEIA ELISA Sets				
TNF-α (Mono/Mono)	Ms	15.6-1000 pg/ml	555268	
TNF-α (Mono/Poly)	Ms	15.6-1000 pg/ml	558874	
TNFR II	Ms	7.8-500 pg/ml	558857	
TNF-α	Hu	7.8-500 pg/ml	555212	
TNFR I	Hu	15.6-1000 pg/ml	550996	
Lymphotoxin-α	Hu	15.6-1000 pg/ml	550995	
TNF-α	Monkey	7.8-500 pg/ml	551493	
TNF-α	Rat	31.3-2000 pg/ml	558870	

DESCRIPTION	SPECIES	ASSAY RANGE	CAT. NO.	NEW
BD OptEIA ELISA Kits				
TNF-α	Ms	31.3-2000 pg/ml	559732	
TNF-α (kit II)	Hu	7.7-500 pg/ml	550610	
TNF-α CL ELISA kit	Hu	1.6-5000 pg/ml	551502	
TNF-α	Rat	31.3-2000 pg/ml	550734	

DESCRIPTION	SPECIES	SIZE	CAT. NO.	NEW
BD ELISPOT Reagents				
TNF-α ELISPOT set	Ms	10 plates	551491	
TNF-α ELISPOT pair	Ms	Abs for 5 plates	551875	
TNF-α ELISPOT set	Hu	10 plates	551446	
TNF-α ELISPOT pair	Hu	Abs for 5 plates	551882	

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NEW Tools for *Drosophila* Proteomics Research

By Rubén Darío Flores-Saaib, Anissa Agadir, Yuerong Zhou, Michael Boyer, and Lisa Stein

In the last fifteen years a unified view of biology has emerged with the discovery that many previously thought dissimilar cellular processes are regulated by similar molecules and combinatorial signals in eukaryotes. Examples include transcription, signal transduction, neurogenesis, hematopoiesis, immune function and protein postranslational modifications.[1]

In the forefront of this revolution in biology, researchers have developed and applied powerful genetic, genomic, and molecular biology methods to study the fruit fly *Drosophila melanogaster*, resulting in some of the most spectacular advances in our understanding of complex eukaryotic biological processes.[2,3] In addition, the evolutionary conservation of the molecular mechanisms regulating metazoan development and its ease of genetic manipulation has made *Drosophila* an attractive model to identify potential new target genes involved in human disease.[4] One of the many advantages of *Drosophila* research is the ability to observe protein localization and protein-protein interactions within the intact organism via antibody immunochemical staining methods.

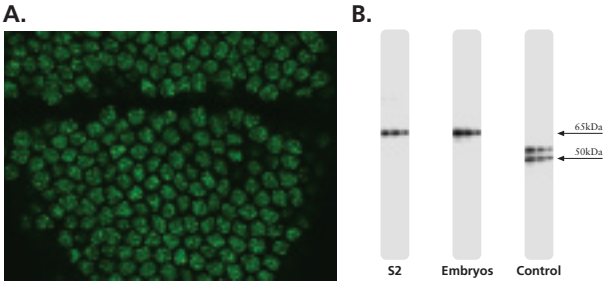

Figure 1. HDAC3 expression in Drosophila. *(A) Scanning confocal microscopy view of the dorsal furrow in a gastrulating embryo stained with anti-HDAC3 mAb. Staining is detected exclusively throughout the nucleus, but more intensively in discrete foci. (B) Western blot analysis of S2 cells lysate (15 ug/lane), 0-12 hours Drosophila embryos (15 ug/lane) or Human Endothelial cells (10 ug/lane) were probed with anti-HDAC3 mAb at concentrations of 2 ug/ml, 1 ug/ml and 0.5 ug/ml.*

Although great strides have been made in the understanding of the genetic hierarchies that regulate biological processes in *Drosophila*, the field of *Drosophila* proteomics is currently hindered by the lack of high quality antibody reagents to a broad spectrum of targets.

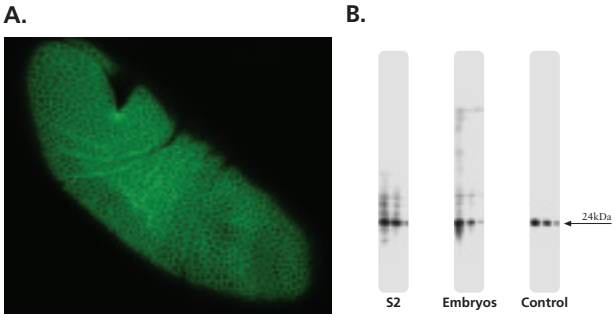

Figure 2. Rab 8 expression in Drosophila. *(A) Scanning confocal microscopy analysis of a stage 7. Anti-Rab8 mAb is labeling membranes, and cytoplasmic "speckles". Nuclei are devoid of staining. (B) Western Blot Analysis. Lysates from S2 cells (15 ug/lane), 0-12 hours Drosophila embryos (15 ug/lane) or Jurkat cells (10 ug/lane) were probed with anti Rab8 at concentrations of 0.05 ug/ml, 0.025 ug/ml, and 0.0125 ug/ml.*

BD Biosciences Pharmingen offers over 1000 high quality monoclonal antibodies against mammalian proteins involved in cell signaling. To test whether these antibodies can be used for *Drosophila* research we first performed a Blast analysis of the mammalian immunogens used to generate our antibodies against the release 3.0 version of the predicted *Drosophila* proteome (www.fruitfly.org). Antibodies made against mammalian immunogens that had high sequence homology to *Drosophila* proteins were then analyzed for cross-reactivity to *Drosophila* proteins and tissues by western blot and immunofluorescence ***(Table 1)***. Antibodies which by western blot recognized an antigen with a close match to the expected molecular weight of the *Drosophila* homologue were chosen for immunoflourescence staining of 0–12 hour whole mount formalin-fixed embryos. Confocal microscopy analysis of the antibody staining allowed us to determine whether the distribution of the antigen corresponds to the expected sub-cellular localization of the *Drosophila* homologue ***(Table 1, Figures 1 and 2)***. These antibodies will allow more extensive and detailed analysis of changes in protein expression in Drosophila research.

Coming soon: purified antibodies to Chaoptin (24B10), Futsch (22C10), Syntaxin (8C3) and Cyclin B (F2F4).

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NEW Tools for *Drosophila* Proteomics Research *(continued from page 19)*

References:

1. Rubin, G.M., et al., Comparative genomics of the eukaryotes. *Science*, 2000. 287(5461): p. 2204-15.
2. The FlyBase database of the Drosophila genome projects and community literature. *Nucleic Acids Res*, 2002. 30(1): p. 106-8.
3. Hekmat-Scafe, D.S., et al., Genome-wide analysis of the odorant-binding protein gene family in Drosophila melanogaster. *Genome Res*, 2002. 12(9): p. 1357-69.
4. Reiter, L.T., et al., A systematic analysis of human disease-associated gene sequences in Drosophila melanogaster. *Genome Res*, 2001. 11(6): p. 1114-25.

Table 1. *Western blot and immunohistochemical analysis of antibodies with cross-reactivity to Drosophila. Blast search was performed with original antigen sequences using the stand-alone blastall program against Drosophila peptide sequences (release 3 from the Berkeley Drosophila Genome Project) with command: blastall -p blastp -d drosophila.aa -m 8 -i ig.txt -o blast.out. The Drosophila protein with the highest homology to the mammalian antigen is listed under the "Drosophila putative homologue" column.*

ANTIBODY DESCRIPTION	DROSOPHILA PUTATIVE HOMOLOGUE	PERCENTAGE HOMOLOGY	EXPECTED MW	OBSERVED MW	IHC
AP50	AP-50	86	50 kDa	50 kDa	+
Arginase I	Arginase	46.49	40 kDa	52 kDa	+
B2 Bradykinin Receptor	Smoothened	83	116 kDa	116 kDa	+
CaM Kinase II	CamKII	91	55 kDa	66 kDa	+
Cathepsin D	CathD	64	42 kDa	42 kDa	+
Desmoglein	DE-Cadherin (Shotgun)	49	169 kDa	~170 kDa	-
Dynamin	Shibine (shi)	41	99 kDa	99 kDa	+
ENC-1	dbo	33	68 kDa	68 kDa	+
Endothelin 1 Receptor	CG8784	35	45 kDa	50 kDa	+
Ets-1	Pointed (pnt)	77	77 kDa	55 kDa	+
FKBP12	Fk506-bp2	75	12 kDa	12 kDa	-
G beta	Gb13F	93	37 kDa	37 kDa	+
Gephyrin	cinnamon (cin)	43	65 kDa	72 kDa	-
gp250/LR11/SorLA/	CG12139	42	217 kDa	> 200 kDa	+
HDAC3	DmHDAC3	46	50 kDa	64 kDa	+
Hsp70	Hsp70	75	70 kDa	70 kDa	+
JNKK1/MKK4	Mkk4	45	47 kDa	47 kDa	-
Numb	numb	38	60 kDa	65 kDa	+
p 50 Dynactin	CG8269	34	41 kDa	41 kDa	+
pan ERK	DmERK-B	50	42 kDa	42 kDa	+
Paxillin	Paxillin (pax)	54	55 kDa	66 kDa	-
PKA RI	Pka-R1	82	42 kDa	42 kDa	-
PKB alpha/Akt	Akt1	61	60 kDa	72 kDa	+
PKC iota	aPKC	73	78 kDa	75 kDa	-
PLCB4/Phospholipase C beta 4	Phospholipase C	33	145 kDa	130 kDa	+
PLK-1	polo	53	66 kDa	60 kDa	+
PP1	Pp1	94	34 kDa	34 kDa	+
Rab11	Rab11	82	24 kDa	24 kDa	-
Rab8	Rab8	68	24 kDa	24 kDa	+
Rac1	Rac1	64	21 kDa	21 kDa	+
Rb2	Rbp2, Rbf	35	96 kDa	>116 kDa	-
Rch-1/Karyopherin alpha 2	Kapa1/3	52	56 kDa	66 kDa	+
SIP1	CG10419	28	28 kDa	28 kDa	+
ZBP-89	Distpached	64	74 kDa	74 kDa	+

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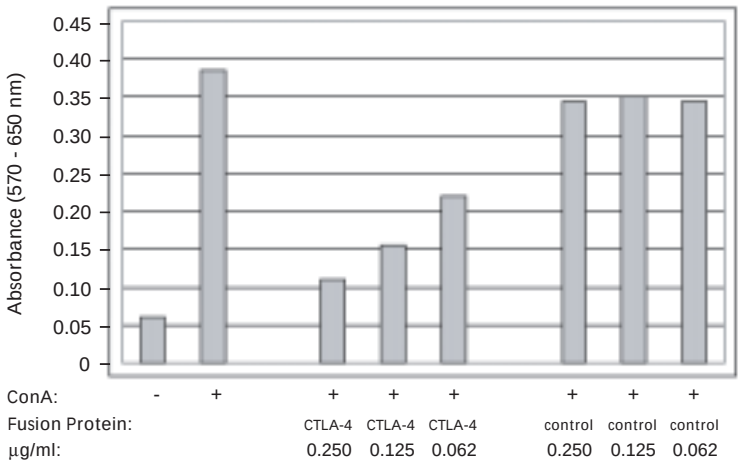
Soluble Fusion Protein for the Study of T-Lymphocyte Costimulation

By Florence Harrod, Ph.D.



Inhibition of ConA-induced proliferation of splenic T lymphocytes by Non-Cytolytic Mouse CTLA-4–IgG Fusion Protein. *C57BL/6 splenocytes were cultured for three days, either with no stimulation or with 4 µg ConA per 10^6 cells, as indicated. The indicated concentrations of either Non-Cytolytic Mouse CTLA-4–IgG Fusion Protein or a control fusion protein were added to ConA-stimulated cells. Cell proliferation was quantitated by the MTT fluorometric assay. The CTLA-4–IgG fusion protein inhibited ConA-induced cell proliferation in a dose-dependent manner, whereas the control fusion protein had little effect.*

Many of the monoclonal antibodies in BD Biosciences portfolio are useful for the study of receptor-ligand interactions when the identities of the receptor and ligand are known. Soluble Recombinant Fusion Proteins are another type of tool for identification of ligands and/or study of the downstream effects of ligand binding. They are especially useful when the ligand is unknown or when a receptor binds multiple ligands.

Non-Cytolytic Mouse CTLA-4–IgG Fusion Protein is particularly useful for investigating the mechanism of T-cell costimulation. It is composed of the extracellular domain of mouse CTLA-4 (CD152) fused to a mutant Fc region of mouse IgG2a which is unable to bind to complement or Fc receptors.[1] In addition to identification of CTLA-4's ligands (B7-1 and B7-2),[1] this soluble protein can block the binding of those ligands to their receptors (CTLA-4 and CD28) and thereby prevents their T-lymphocyte regulatory actions.[1,2,3]

References:

1. Steurer, W., P.W. Nickerson, A.W. Steele, J. Steiger, X.X. Zheng, and T.B. Strom. 1995. Ex vivo coating of islet cell allografts with murine CTLA4/Fc promotes graft tolerance. *J. Immunol.* 155: 1165 – 1174.
2. Tivol, E.A., S.D. Boyd, S. McKeon, F. Borriello, P. Nickerson, T.B. Strom, and A.H. Sharpe. 1997. CTLA4Ig prevents lymphoproliferation and fatal multiorgan tissue destruction in CTLA-4-deficient mice. *J. Immunol.* 158: 5091 – 5094.
3. Gonzalo, J.-A., T. Delaney, J. Corcoran, A. Goodearl, J.C. Gutierrez-Ramos ,and A.J. Coyle. 2001. The related molecules CD28 and inducible costimulator deliver both unique and complementary signals required for optimal T cell activation. *J. Immunol.* 166: 1 – 5.

DESCRIPTION	FORMAT	SIZE	CAT. NO.
Non-Cytolytic Mouse CTLA-4 - IgG Fusion Protein	NA/LE	0.25 mg	**552132**
Non-Cytolytic Mouse CTLA-4 - IgG Fusion Protein	NA/LE	0.5 mg	**552133**

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Analysis of Granulocytes & Monocytes in Mouse Peripheral Blood

By Lori Gillette, Florence Harrod, Ph.D., and Andrea Nguyen

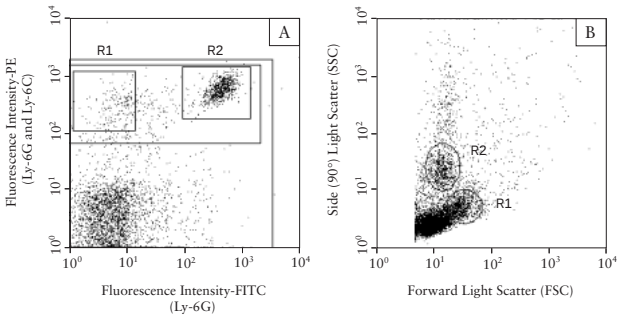
BD Biosciences Pharmingen offers two antibodies to mouse Ly-6G, one of which also recognizes Ly-6C, for distinguishing granulocyte and monocyte cell populations in mouse.

Clone **1A8** antibody reacts with Ly-6G, a 21-25–kDa GPI-anchored protein.[1] In the periphery, it is expressed on granulocytes.[1,2]

Clone **RB6-8C5** antibody reacts with Ly-6G, previously known as the myeloid differentiation antigen Gr-1, a 21-25–kDa GPI-anchored protein.[1] Weak reactivity with Ly-6C-transfected EL4J cells has also been detected.[1] In the periphery, RB6-8C5 antibody recognizes granulocytes (neutrophils and eosinophils) and monocytes.[2,3,4,5]

References:

1. Fleming, T.J., M.L. Fleming, and T.R. Malek. 1993. Selective expression of Ly-6G on myeloid lineage cells in mouse bone marrow. RB6-8C5 mAb to granulocyte-differentiation antigen (Gr-1) detects members of the Ly-6 family. *J. Immunol.* 151: 2399 – 2408.
2. Lagasse, E., and I.L. Weissman. 1996. Flow cytometric identification of murine neutrophils and monocytes. *J. Immunol.* Methods 197: 139 – 150.
3. Tepper, R.I., R.L. Coffman, and P. Leder. 1992. An eosinophil-dependent mechanism for the antitumor effect of interleukin-4. *Science* 257: 548 – 551.



Representative staining of peripheral blood leukocytes with PE-conjugated antibody RB6-8C5. *C57BL/6 whole blood was stained with PE-conjugated RB6-8C5 (anti-Ly-6G and Ly-6C, Cat. No. 553128) and FITC-conjugated 1A8 (anti-mouse Ly-6G, Cat. No. 551460) monoclonal antibodies in the presence of Mouse BD Fc Block™ purified anti-mouse CD16/CD32, mAb 2.4G2 (Cat. No. 553141/553142, Panel A). Erythrocytes were lysed (BD PharM Lyse™, Cat. No. 555899) and non-viable leukocytes were excluded by staining with propidium iodide. Panel A demonstrates that mAb 1A8 stains the RB6-8C5^{hi} population, corresponding to Ly-6G-expressing granulocytes; whereas, the RB6-8C5^{lo} population is 1A8-negative and corresponds to Ly-6C-expressing lymphocytes and monocytes. Backgating of the RB6-8C5^{+}/1A8^{-} population (R1) onto the light-scatter profile (Panel B) indicates that this population falls within the monocyte region of the light-scatter profile. The RB6-8C5^{+}/1A8^{+} positive population (R2) falls within the granulocyte region of the light-scatter profile (Panel B). Flow cytometry was performed on a BD FACSCalibur™ Flow Cytometer.*

4. Conlan, J.W., and R.J. North. 1994. Neutrophils are essential for early anti-Listeria defense in the liver, but not in the spleen or peritoneal cavity, as revealed by a granulocyte-depleting monoclonal antibody. *J. Exp. Med*. 179: 259 – 268.
5. BD Biosciences Pharmingen. Unpublished results.

SPECIFICITY	FORMAT	APP	SPECIES	CLONE	CAT. NO.
Ly-6G and Ly-6C	Purified	IHC	Ms	RB6-8C5	550291
Ly-6G and Ly-6C	Purified	Cyt, FCM, IP/WB	Ms	RB6-8C5	557445
Ly-6G and Ly-6C	Purified	Cyt, FCM, IP/WB	Ms	RB6-8C5	553123
Ly-6G and Ly-6C	NA/LE	Cyt, FCM	Ms	RB6-8C5	553122
Ly-6G and Ly-6C	Biotin	Sep, FCM	Ms	RB6-8C5	553124
Ly-6G and Ly-6C	Biotin	Sep, FCM	Ms	RB6-8C5	553125
Ly-6G and Ly-6C	FITC	FCM	Ms	RB6-8C5	553126
Ly-6G and Ly-6C	FITC	FCM	Ms	RB6-8C5	553127
Ly-6G and Ly-6C	PE	FCM	Ms	RB6-8C5	553128
Ly-6G and Ly-6C	APC	FCM	Ms	RB6-8C5	553129
Ly-6G and Ly-6C	PerCP-Cy5.5	FCM	Ms	RB6-8C5	552093
Ly-6G and Ly-6C	PerCP-Cy5.5	FCM	Ms	RB6-8C5	552987
Ly-6G	Purified	FCM, IHC, IP	Ms	1A8	551459
Ly-6G	FITC	FCM	Ms	1A8	551460
Ly-6G	PE	FCM	Ms	1A8	551461

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2003 Catalog Product Supplement for BD Biosciences

BD Biosciences Immunocytometry Systems

Viability Testing

DESCRIPTION	APPS	REG	FORMAT	SIZE	CAT. NO.	PRICE
BD Cell Viability Kit	FCM	RUO (GMP)	Thiazole Orange/ Propidium Iodide	100 tests	**349493**	$145
BD Cell Viability Kit with BD Liquid Counting Beads	FCM	RUO (GMP)	Thiazole Orange/ Propidium Iodide	100 tests	**349480**	$395

BD FastImmune Antibody Reagents

DESCRIPTION	REACT	CLONE	APPS	REG	FORMAT	SIZE	CAT. NO.	PRICE
IFN-γ	Hu		FCM	RUO (GMP)	APC	100 tests	**341117**	$510
IL-2	Hu		FCM	RUO (GMP)	APC	100 tests	**341116**	$510
CD63/CD123/HLA-DR	Hu	H5C6/9F5/L243	FCM	RUO (GMP)	FITC/PE/PerCP	50 tests	**341068**	$620

Antibodies to Human Cell Surface Molecules

ASR Reagents

DESCRIPTION	REACT	CLONE	ISOTYPE	REG	FORMAT	CAT. NO.	PRICE
CD3	Hu	SK7	Mouse IgG_1	ASR	PE-Cy7	**341101**	$550
				ASR	APC-Cy7	**341100**	$550
CD4	Hu	SK3	Mouse IgG_1	ASR	APC-Cy7	**341105**	$550
CD5	Hu	L17F12	Mouse IgG_{2a}	ASR	PerCP-Cy5.5	**341099**	$310
CD10	Hu	HI10a	Mouse IgG_1	ASR	PE-Cy7	**341102**	$550
CD14	Hu	MφP9	Mouse IgG_{2b}	ASR	APC-Cy7	**333948**	$550
CD19	Hu	4G7	Mouse IgG_1	ASR	PE-Cy7	**341103**	$550
CD33	Hu	P67.6	Mouse IgG_1	ASR	PE-Cy7	**333949**	$550
CD117	Hu	104D2	Mouse IgG_1	ASR	APC	**341106**	$510
				ASR	PerCP-Cy5.5	**333947**	$510
CD138	Hu	MI15	Human IgG_1	ASR	FITC	**347205**	$235
				ASR	PE	**347206**	$265
				ASR	APC	**347207**	$510
				ASR	PerCP-Cy5.5	**341097**	$310
Anti-Kappa	Hu	TB 28-2	Mouse IgG_1	ASR	APC	**341098**	$510

RUO Reagents

DESCRIPTION	REACT	CLONE	ISOTYPE	APPS	REG	FORMAT	SIZE	CAT. NO.	PRICE
CD3	Hu	SK7	Mouse IgG_1	FCM	RUO (GMP)	APC-Cy7	100 tests	**341090**	$550
				FCM	RUO (GMP)	PE-Cy7	100 tests	**341091**	$550
CD4	Hu	SK3	Mouse IgG_1	FCM	RUO (GMP)	APC-Cy7	100 tests	**341095**	$550
CD5	Hu	L17F12	Mouse IgG_{2a}	FCM	RUO (GMP)	PerCP-Cy5.5	50 tests	**341089**	$310
CD10	Hu	HI10a	Mouse IgG_1	FCM	RUO (GMP)	PE-Cy7	100 tests	**341092**	$550
CD14	Hu	MφP9	Mouse IgG_{2b}	FCM	RUO (GMP)	APC-Cy7	100 tests	**333945**	$550
CD19	Hu	4G7	Mouse IgG_1	FCM	RUO (GMP)	PE-Cy7	100 tests	**341093**	$550
CD33	Hu	P67.6	Mouse IgG_1	FCM	RUO (GMP)	PE-Cy7	100 tests	**333946**	$550
CD117	Hu	104D2	Mouse IgG_1	FCM	RUO (GMP)	APC	100 tests	**341096**	$510
				FCM	RUO (GMP)	PerCP-Cy5.5	50 tests	**333944**	$310
CD138	Hu	MI15	Human IgG_1	FCM	RUO (GMP)	FITC	50 tests	**347191**	$235
				FCM	RUO (GMP)	PE	50 tests	**347192**	$265
				FCM	RUO (GMP)	PerCP-Cy5.5	50 tests	**341087**	$310
				FCM	RUO (GMP)	APC	100 tests	**347193**	$510
Anti-Kappa	Hu	TB 28-2	Mouse IgG_1	FCM	RUO (GMP)	APC	100 tests	**341088**	$510

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

ASR: *Analyte Specific Reagent. Analytical and performance characteristics are not established.*

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Product Supplement *(continued from page 23)*

BD Biosciences Pharmingen

Adhesion Molecules

Antibodies to Human Adhesion Molecules - Others (Human)

DESCRIPTION	REACT	CLONE	ISOTYPE	APPS	FORMAT	SIZE	CAT. NO.	PRICE
CD134 (OX40)	Hu	ACT35	Mouse IgG_1, κ	FCM	PE-Cy5 (BD Cy-Chrome™)	100 tests	**551500**	$295

BD™ Cytometric Bead Array

BD Cytometric Bead Array

DESCRIPTION	REACT	APPS	SIZE	CAT. NO.	PRICE
Human Inflammation Standards	Hu	FCM	1 vial	**552932**	$175
Mouse Th1/Th2 Standards	Ms	FCM	1 vial	**552967**	$175

** Please see **page 9** for BD Cytometric Bead Array Kits available form the Custom products and Services Group.*

BD™ ELISPOT

Human ELISPOT Reagents

DESCRIPTION	REACT	APPS	SIZE	CAT. NO.	PRICE
Human Granzyme B Kit	Hu	ELISPOT	2 plates	**552573**	$695
Human Granzyme B Set	Hu	ELISPOT	10 plates	**552572**	$850
Human IL-4 Kit	Hu	ELISPOT	2 plates	**552141**	$695

Mouse ELISPOT Reagents

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

Rat ELISPOT Reagents

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

BD OptEIA™

BD OptEIA CL Chemiluminescent ELISA Kits

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

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Cell Biology / Cell Signaling

Accessory Reagents

DESCRIPTION	REACT	CLONE	ISOTYPE	APPS	FORMAT	SIZE	CAT. NO.	PRICE
Goat Anti-Rabbit Ig	Rab	Poly	Goat Ig	ELISA, WB	HRP	1 mg	**610084**	$105

Antibodies

DESCRIPTION	REACT	CLONE	ISOTYPE	APPS	FORMAT	SIZE	CAT. NO.	PRICE
ACT1	Hu, Rat	Poly	Rabbit Ig	WB	Serum	100 µl	**552816**	$210
Apaf-1	Hu, Ms	Poly	Rabbit Ig	WB	Serum	100 µl	**552813**	$210
Cdc25C	Hu	TC113	Mouse IgG_1	IF, IP, WB	Purified	150 µg	**550921**	$295
Caspase-3	Hu, Ms, Rat	Poly	Rabbit Ig	WB	Serum	100 µl	**552785**	$210
Caspase-10	Hu, Ms, Rat	Poly	Rabbit	WB	Serum	100 µl	**552810**	$210
Caspase-7	Hu, Ms, Rat	Poly	Rabbit	WB	Serum	100 µl	**552815**	$210
Cdk1/Cdk2	Hu	AN21.2	Mouse IgG_{2a}	WB	Purified	150 µg	**551525**	$295
CLP-36	Dog, Hu, Ms, Rat	51	Mouse IgG1	IF, WB	Purified	50 µg	**612336**	$175
CLP-36	Dog, Hu, Ms, Rat	51	Mouse IgG1	IF, WB	Purified	150 µg	**612337**	$295
Caspase-3, Active Form, ELISA Set (CPP32)	Hu	19, C92-605	Mouse IgG2a, Rabbit	ELISA	Set	5 96-well plates	**550930**	$490
DsRed		Poly	Rabbit Ig	WB	Serum	100 µl	**552941**	$210
β-Dystroglycan (pY892)	Hu, Ms	27.1	Mouse IgG1	IF, WB	Purified	50 µg	**612524**	$225
β-Dystroglycan (pY892)	Hu, Ms	27.1	Mouse IgG1	IF, WB	Purified	150 µg	**612525**	$400
E2	Hu, Ms	Poly	Rabbit Ig	WB	Serum	100 µl	**552735**	$210
c-IAP1	Hu	F30-2285	Mouse IgG_1, κ	WB	Purified	50 µg	**552782**	$175
c-IAP1	Hu	F30-2285	Mouse IgG_1, κ	WB	Purified	150 µg	**552783**	$295
Integrin β3 (pY759)	Hu	7a	Mouse IgG1	WB	Purified	50 µg	**612528**	$225
Integrin β3 (pY759)	Hu	7a	Mouse IgG1	WB	Purified	150 µg	**612529**	$400
JNK (pT183/pY185)	Hu, Ms, Rat	41	Mouse IgG1	FCM, WB	Purified	50 µg	**612540**	$225
JNK (pT183/pY185)	Hu, Ms, Rat	41	Mouse IgG1	FCM, WB	Purified	150 µg	**612541**	$400
Lck (pY505)	Hu, Ms, Rat	4	Mouse IgG1	FCM, WB	Purified	50 µg	**612390**	$225
Lck (pY505)	Hu, Ms, Rat	4	Mouse IgG1	FCM, WB	Purified	150 µg	**612391**	$400
eNOS (pS1177)	Hu	19	Mouse IgG1	FCM, WB	Purified	50 µg	**612392**	$225
eNOS (pS1177)	Hu	19	Mouse IgG1	FCM, WB	Purified	150 µg	**612393**	$400
PARP, Cleaved Form (Asp214)	Ms	F21-852	Mouse IgG_1, κ	FCM, IP, WB	PE	100 tests	**552934**	$395
PKD	Hu, Ms, Rat	Poly	Rabbit Ig	WB	Serum	100 µl	**552817**	$210
PKD (S916)	Hu, Ms	Poly	Rabbit Ig	WB	Serum	100 µl	**552818**	$210
p35	Bv	Poly	Rabbit Ig	WB	Serum	100 µl	**552812**	$210
p120 Catenin (pY96)	Hu, Ms	25a	Mouse IgG1	WB	Purified	50 µg	**612534**	$225
p120 Catenin (pY96)	Hu, Ms	25a	Mouse IgG1	WB	Purified	150 µg	**612535**	$400
p120 Catenin (pY228)	Hu, Ms	21a	Mouse IgG1	IF, WB	Purified	50 µg	**612536**	$225
p120 Catenin (pY228)	Hu, Ms	21a	Mouse IgG1	IF, WB	Purified	150 µg	**612537**	$400
p120 Catenin (pY280)	Hu	18	Mouse IgG1	WB	Purified	50 µg	**612538**	$225
p120 Catenin (pY280)	Hu	18	Mouse IgG1	WB	Purified	150 µg	**612539**	$400
Phospholipase Cγ (pY783)	Hu	27	Mouse IgG1	WB	Purified	50 µg	**612464**	$225
Phospholipase Cγ (pY783)	Hu	27	Mouse IgG1	WB	Purified	150 µg	**612465**	$400
$PKA_{RIIβ}$ (pS114)	Hu, Ms, Rat	47	Mouse IgG1	WB	Purified	50 µg	**612550**	$225
$PKA_{RIIβ}$ (pS114)	Hu, Ms, Rat	47	Mouse IgG1	WB	Purified	150 µg	**612551**	$400
Phosphoserine	Hu, Rat	19	Mouse IgG1	IF, WB	Purified	50 µg	**612546**	$225

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Product Supplement (continued from page 25)

Cell Biology / Cell Signaling

Antibodies (cont'd)

DESCRIPTION	REACT	CLONE	ISOTYPE	APPS	FORMAT	SIZE	CAT. NO.	PRICE
Phosphoserine	Hu, Rat	19	Mouse IgG1	IF, WB	Purified	150 µg	**612547**	$400
Phosphoserine/threonine	Hu, Rat	22a	Mouse IgG1	IF, WB	Purified	50 µg	**612548**	$225
Phosphoserine/threonine	Hu, Rat	22a	Mouse IgG1	IF, WB	Purified	150 µg	**612549**	$400
Stat3 (pS727)	Hu, Ms, Rat	49	Mouse IgG1	IF, WB	Purified	50 µg	**612542**	$225
Stat3 (pS727)	Hu, Ms, Rat	49	Mouse IgG1	IF, WB	Purified	150 µg	**612543**	$400
TLR3	Hu	F28-1707	Mouse IgG_1, κ	WB	Purified	50 µg	**552753**	$175
TLR3	Hu	F28-1707	Mouse IgG_1, κ	WB	Purified	150 µg	**552752**	$295
T Cell PTPase	Hu	Poly	Rabbit Ig	WB	Purified	100 µl	**552763**	$210

Antibody Sampler Kits

DESCRIPTION	APPS	SIZE	CAT. NO.	PRICE
EGFR Activation Sampler Kit	WB	1 Kit	**612476**	$415
MAP Kinase Activation Sampler Kit	WB	1 Kit	**612544**	$415
Stat Activation Sampler Kit	WB	1 Kit	**612477**	$415

DNA Damage Reagents/Kits

DESCRIPTION	REACT	SIZE	CAT. NO.	PRICE
Mouse Microflow® Basic Kit	Ms	60 tests	**552728**	$3300
Mouse Microflow® Plus Kit	Ms	60 tests	**552730**	$1350
Rat Microflow® Basic Kit	Rat	60 tests	**552729**	$3300
Rat Microflow® Plus Kit	Rat	60 tests	**552731**	$1350

Phospho-Specific Antibodies

DESCRIPTION	REACT	CLONE	ISOTYPE	APPS	FORMAT	SIZE	CAT. NO.	PRICE
β-Dystroglycan (pY892)	Hu, Ms	27.1	Mouse IgG1	IF, WB	Purified	50 µg	**612524**	$225
β-Dystroglycan (pY892)	Hu, Ms	27.1	Mouse IgG1	IF, WB	Purified	150 µg	**612525**	$400
Integrin β3 (pY759)	Hu	7a	Mouse IgG1	WB	Purified	50 µg	**612528**	$225
Integrin β3 (pY759)	Hu	7a	Mouse IgG1	WB	Purified	150 µg	**612529**	$400
JNK (pT183/pY185)	Hu, Ms, Rat	41	Mouse IgG1	FCM, WB	Purified	50 µg	**612540**	$225
JNK (pT183/pY185)	Hu, Ms, Rat	41	Mouse IgG1	FCM, WB	Purified	150 µg	**612541**	$400
Lck (pY505)	Hu, Ms, Rat	4	Mouse IgG1	FCM, WB	Purified	50 µg	**612390**	$225
Lck (pY505)	Hu, Ms, Rat	4	Mouse IgG1	FCM, WB	Purified	150 µg	**612391**	$400
eNOS (pS1177)	Hu	19	Mouse IgG1	FCM, WB	Purified	50 µg	**612392**	$225
eNOS (pS1177)	Hu	19	Mouse IgG1	FCM, WB	Purified	150 µg	**612393**	$400
p120 Catenin (pY96)	Hu, Ms	25a	Mouse IgG1	WB	Purified	50 µg	**612534**	$225
p120 Catenin (pY96)	Hu, Ms	25a	Mouse IgG1	WB	Purified	150 µg	**612535**	$400
p120 Catenin (pY228)	Hu, Ms	21a	Mouse IgG1	IF, WB	Purified	50 µg	**612536**	$225
p120 Catenin (pY228)	Hu, Ms	21a	Mouse IgG1	IF, WB	Purified	150 µg	**612537**	$400
p120 Catenin (pY280)	Hu	18	Mouse IgG1	WB	Purified	50 µg	**612538**	$225
p120 Catenin (pY280)	Hu	18	Mouse IgG1	WB	Purified	150 µg	**612539**	$400
Phospholipase Cγ (pY783)	Hu	27	Mouse IgG1	WB	Purified	50 µg	**612464**	$225
Phospholipase Cγ (pY783)	Hu	27	Mouse IgG1	WB	Purified	150 µg	**612465**	$400

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Cell Biology / Cell Signaling

Phospho-Specific Antibodies (cont'd)

DESCRIPTION	REACT	ISOTYPE	APPS	FORMAT	SIZE	CAT. NO.	PRICE
$PKA_{RII\beta}$ (pS114)	Hu, Ms, Rat 47	Mouse IgG1	WB	Purified	50 µg	**612550**	$225
$PKA_{RII\beta}$ (pS114)	Hu, Ms, Rat 47	Mouse IgG1	WB	Purified	150 µg	**612551**	$400
Stat3 (pS727)	Hu, Ms, Rat 49	Mouse IgG1	IF, WB	Purified	50 µg	**612542**	$225
Stat3 (pS727)	Hu, Ms, Rat 49	Mouse IgG1	IF, WB	Purified	150 µg	**612543**	$400

Positive Control Lysates

DESCRIPTION	APPS	TISSUE TYPE	SIZE	CAT. NO.	PRICE
PC12 Cell Lysate	WB	Neurobastoma	500 µg	**611454**	$85
SW-13 Cell Lysate	WB	Adenocarcinoma	500 µg	**611475**	$85

Cytokines, Chemokines, and Inflammatory Mediators

Antibodies to human cytokines, chemokines and inflammatory mediators - Inflammatory mediators and their receptors

DESCRIPTION	REACT	CLONE	ISOTYPE	APPS	FORMAT	SIZE	CAT. NO.	PRICE
C1qRp (CDw93)	Hu	R139	Mouse IgG_{2b},κ	FCM, IP, Neu, WB	NA/LE	0.1 mg	**552954**	$395
CD21 (CR2)	Hu	1048	Mouse IgG_1, κ	FCM	Purified	0.1 mg	**552727**	$120

Antibodies to human cytokines, chemokines and inflammatory mediators - Cytokines and their receptors

DESCRIPTION	REACT	CLONE	ISOTYPE	APPS	FORMAT	SIZE	CAT. NO.	PRICE
IL-18 Receptor	Hu	H44	Mouse IgG_1, κ	FCM	Purified	0.1 mg	**552951**	$150
Leucotriene B4 Receptor (hBLTR-1)	Hu	203/14F11	Mouse IgG_1	FCM	Biotin	100 tests	**552835**	$215
				FCM	PE	100 tests	**552836**	$260

Antibodies to human cytokines, chemokines and inflammatory mediators - Inflammatory mediators and their receptors

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

Antibodies to mouse cytokines, chemokines and inflammatory mediators - Cytokines and their receptors

DESCRIPTION	REACT	CLONE	ISOTYPE	APPS	FORMAT	SIZE	CAT. NO.	PRICE
CD124 (IL-4 Receptor)	Ms	mIL4R-M2	Rat IgG_{2a},κ	ELISA, FCM	Purified	0.5 mg	**552952**	$275
IL-12 Receptor β_2	Ms	HAM10B9	Hamster IgG	FCM	Purified	0.1 mg	**552819**	$120

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Product Supplement (continued from page 27)

Human Cell Surface Molecules

Antibodies to Human Leukocytes

DESCRIPTION	REACT	CLONE	ISOTYPE	APPS	FORMAT	SIZE	CAT. NO.	PRICE
CD21 (CR2)	Hu	1048	Mouse IgG_1, κ	FCM	Purified	0.1 mg	**552727**	$120
CD66	Hu	COL-1	Mouse IgG_{2a}, κ	FCM	Purified	0.1 mg	**551477**	$120
CD66 (CEA, carcinoembryonic antigen)	Hu	B1.1/CD66	Mouse IgG_{2a},κ	FCM	PE	100 tests	**551480**	$260
CD108	Hu	KS-2	Mouse IgG_{2a}, κ	FCM	Purified	0.1 mg	**552423**	$120
CD134 (OX40)	Hu	ACT35	Mouse IgG_1, κ	FCM	PE-Cy5 (BD Cy-Chrome™)	100 tests	**551500**	$295
CD138	Hu	Mi15	Mouse IgG_1, κ	FCM	PE	100 tests	**552723**	$230
CD160	Hu	BY55	Mouse IgM, κ	FCM	Purified	0.1 mg	**551887**	$120
				FCM	FITC	100 tests	**551888**	$230
CD172a/b (SIRPα/β)	Hu	SE5A5	Mouse IgG_1, κ	FCM	PE	100 tests	**552722**	$260
CD172b (SIRPβ)	Hu	B4B6	Mouse IgG_1, κ	FCM	PE	100 tests	**552602**	$260
CD180	Hu	G28-8	Mouse IgG_1, κ	FCM	Purified	0.1 mg	**551890**	$120
CD229	Hu	HLy9.1.25	Mouse IgG_1	FCM	Purified	0.1 mg	**552751**	$120
CD231 (SN1)	Hu	H1-A12	Mouse IgG_1	FCM	FITC	100 tests	**551897**	$230

Antibodies to Human MHC Antigens

DESCRIPTION	REACT	CLONE	ISOTYPE	APPS	FORMAT	SIZE	CAT. NO.	PRICE
TAP2	Hu	TAP2.17	Mouse IgG_1, κ	FCM	FITC Set	100 tests	**551293**	$310

Miscellaneous Antibodies

DESCRIPTION	REACT	CLONE	ISOTYPE	APPS	FORMAT	SIZE	CAT. NO.	PRICE
Basophils	Hu	Bsp-1	Mouse IgM,κ	FCM	Purified	0.1 mg	**552754**	$120
Invariant NK T	Hu	6B11	Mouse IgG_1	FCM	Purified	0.1 mg	**552824**	$120
CMRF-56	Hu	CMRF-56	Mouse IgG_1, κ	FCM	Purified	0.1 mg	**551534**	$120

Immunoglobulin/2nd Step Reagents

Antibodies to Mouse Immunoglobulin Isotypes

DESCRIPTION	REACT	CLONE	ISOTYPE	APPS	FORMAT	SIZE	CAT. NO.	PRICE
Mouse IgM	Ms	R6-60.2	Rat (LOU) IgG_{2a}, κ	FCM	PE-Cy7	0.1 mg	**552867**	$325

BD™ DimerX

DESCRIPTION	REACT	CLONE	ISOTYPE	APPS	FORMAT	SIZE	CAT. NO.	PRICE
Mouse CD1d	Ms		Mouse IgG_1, λ	Peptide Presentation	Purified	0.25 mg	**557599**	$550

BD™ IMag

DESCRIPTION	REACT	CLONE	ISOTYPE	APPS	FORMAT	SIZE	CAT. NO.	PRICE
Anti-Human CD3 Particles-DM*	Hu	HIT3a	Mouse IgG_{2a}, κ	Sep	BD IMag-DM	5.0 ml	**552593**	$450

**For use with the BD IMagnet (552311)*

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Mouse Cell Surface Molecules

Antibodies to Mouse Leukocytes and Related Cells

DESCRIPTION	REACT	CLONE	ISOTYPE	APPS	FORMAT	SIZE	CAT. NO.	PRICE
CD3e (CD3 ε chain)	Ms	145-2C11	Armenian Hamster IgG1, κ	FCM	PE-Cy7	0.1 mg	**552774**	$325
CD4 (L3T4)	Ms	RM4-5	Rat (DA) IgG_{2a}, κ	FCM	PE-Cy7	0.1 mg	**552775**	$325
CD8a (Ly-2)	Ms	53-6.7	Rat (LOU/Ws1/M) IgG_{2a}, κ	FCM	PE-Cy7	0.1 mg	**552877**	$325
CD11b (Integrin α_M chain)	Ms, Hu	M1/70	Rat (DA) IgG_{2b}, κ	FCM	PE-Cy7	0.1 mg	**552850**	$325
CD19	Ms	1D3	Rat (Lewis) IgG_{2a}, κ	FCM	PE-Cy7	0.1 mg	**552854**	$325
CD21/CD35 (CR2/CR1)	Ms	7G6	Rat (SD) IgG_{2b}, κ	FCM	PE	0.2 mg	**552957**	$335
CD25 (IL-2 Receptor α chain, p55)	Ms	PC61	Rat (Outbred OFA) IgG_1, λ	FCM	PE-Cy7	0.1 mg	**552880**	$325
CD36 (Scavenger Receptor)	Ms, Rat	CRF D-2712	Mouse IgA, κ	FCM, FA, IF, IP	Purified	0.5 mg	**552544**	$275
CD42d (Platelet Glycoprotein V)	Ms, Rat	1C2	Armenian Hamster IgG3, λ	FCM, IP, IHC (Fr), ICC, IF	Purified	0.5 mg	**552992**	$265
CD45 (Leukocyte Common Antigen, Ly-5)	Ms	30-F11	Rat (LOU/Ws1/M) IgG_{2b}, κ	FCM	PE-Cy7	0.1 mg	**552848**	$325
CD45.2	Ms	104	Mouse (SJL) IgG_{2a}, κ	FCM	PerCP-Cy5.5	0.1 mg	**552950**	$325
CD45R/B220	Hu, Ms	RA3-6B2	Rat IgG_{2a}, κ	FCM	PerCP-Cy5.5	0.1 mg	**552771**	$325
				FCM	PE-Cy7	0.1 mg	**552772**	$325
CD69 (Very Early Activation Antigen)	Ms	H1.2F3	Armenian Hamster IgG1, λ	FCM	PE-Cy7	0.1 mg	**552879**	$325
CD124 (IL-4 Receptor)	Ms	mIL4R-M2	Rat IgG_{2a}, κ	ELISA, IP	Purified	0.5 mg	**552952**	$275
CD157 (BP-3 Alloantigen)	Ms	BP-3	Mouse (*Mus spretus*) IgG_{2b}, κ	FCM	PE	0.1 mg	**552814**	$275
Dendritic Cells	Ms	33D1	Rat (SD) IgG_{2b}, κ	FCM	Biotin	0.5 mg	**552776**	$285
				FCM	PE	0.2 mg	**557578**	$295
F4/80-Like Receptor (FIRE)	Ms	6F12	Rat IgG_{2a}, κ	FCM, IHC (Fr)	Purified	0.5 mg	**552958**	$275
Ly-6G and Ly-6C (Gr-1)	Ms	RB6-8C5	Rat IgG_{2b}, κ	FCM	PE-Cy7	0.02 mg	**552985**	$250
NK-1.1 (NKR-P1B and NKR-P1C)	Ms	PK136	Mouse IgG_{2a}, κ	FCM	PE-Cy7	0.1 mg	**552878**	$325
Notch1	Ms, Hu	mN1A	Mouse IgG_1, κ	IC/FCM	PE	0.1 mg	**552768**	$205

Antibodies to Mouse T-Cell Receptors (TCR)

DESCRIPTION	REACT	CLONE	ISOTYPE	APPS	FORMAT	SIZE	CAT. NO.	PRICE
Vδ 4 TCR	Ms	GL2	Armenian Hamster IgG2, κ	FCM	FITC	0.1 mg	**552143**	$95

Non-Human Primate

Antibodies for Non-Human Primate (NHP) Research

DESCRIPTION	REACT	CLONE	ISOTYPE	APPS	FORMAT	SIZE	CAT. NO.	PRICE
CD45	Cyno, Rhe	T	Mouse IgG_1, κ	FCM	PerCP-Cy5.5	50 tests	**552724**	$315
CCR6	Cyno, Rhe	11A9	Mouse IgG_1, κ	FCM	PE	50 tests	**551773**	$140
HLA-DR	Bab, Cyno, Rhe	G46-6	Mouse IgG_{2a}, κ	FCM	PerCP-Cy5.5	50 tests	**552764**	$315
IL-16	Cyno, Rhe	14.1	Mouse IgG_{2a}, κ	IC/FCM	PE	50 tests	**551471**	$140
				IC/FCM	APC	50 tests	**551472**	$170

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Product Supplement (continued from page 29)

Non-Human Primate

Mouse Immunoglobulin Isotype Controls

DESCRIPTION	REACT	CLONE	ISOTYPE	APPS	FORMAT	SIZE	CAT. NO.	PRICE
Mouse IgG_{2a}, κ (Specific for TNP)		G155-178	Mouse (BALB/c) IgG_{2a}, κ	FCM	PerCP-Cy5.5	50 tests	**552577**	$150

Other Non-human Species

Antibodies to Pig Leukocytes

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

Rat Cell Surface Molecules

Antibodies to Rat Leukocytes and Related Cells

DESCRIPTION	REACT	CLONE	ISOTYPE	APPS	FORMAT	SIZE	CAT. NO.	PRICE
CD36 (Scavenger Receptor)	Ms, Rat	CRF D-2712	Mouse IgA, κ	FCM, ICC, IF, IP	Purified	0.5 mg	**552544**	$275
Myeloid Lineage	Rat	OX-82	Mouse (BALB/c) IgG_1, κ	FCM	Purified	0.1 mg	**552130**	$95
RT6.1	Rat	P4/16	Rat (AUG a-PVG) IgG_{2b}, κ	FCM	Purified	0.1 mg	**552725**	$95

Isotype Controls

Hamster immunoglobulin isotype controls

DESCRIPTION	CLONE	ISOTYPE	APPS	FORMAT	SIZE	CAT. NO.	PRICE
Hamster IgG3, κ (anti-KLH)	E36-239	Armenian Hamster IgG3, κ	ELISA, FCM, ICtrl	PE	0.1 mg	**552144**	$165
Hamster IgG_1, κ (anti-TNP)	A19-3	Armenian Hamster IgG1, κ	FCM	PE-Cy7	0.1 mg	**552811**	$205

Mouse immunoglobulin isotype controls

DESCRIPTION	CLONE	ISOTYPE	APPS	FORMAT	SIZE	CAT. NO.	PRICE
Mouse IgG_{2a}, κ (ant-TNP)	G155-178	Mouse (BALB/c) IgG_{2a}, κ	FCM	PE-Cy7	0.1 mg	**552868**	$205
			FCM	PerCP-Cy5.5	0.1 mg	**550927**	$250
			FCM	PerCP-Cy5.5	50 tests	**552577**	$150

Rat immunoglobulin isotype controls

DESCRIPTION	CLONE	ISOTYPE	APPS	FORMAT	SIZE	CAT. NO.	PRICE
Rat IgG_1, λ (anti-KLH)	A110-1	Rat (LOU) IgG_1, λ	FCM	PE-Cy7	0.1 mg	**552869**	$205
Rat IgG_{2a}, κ	R35-95	Rat (LOU) IgG_{2a}, κ	FCM	APC-Cy7	0.1 mg	**552770**	$205
			FCM	PE-Cy7	0.1 mg	**552784**	$205
Rat IgG_{2b}, κ	A95-1	Rat (LOU) IgG_{2b}, κ	FCM	APC-Cy7	0.1 mg	**552773**	$205
			FCM	PE-Cy7	0.1 mg	**552849**	$205

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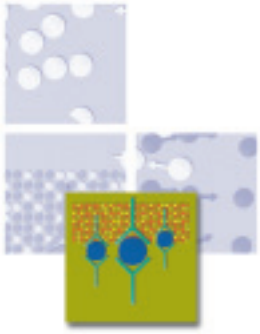

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Where We'll Be Winter & Spring 2003

When	What	Where
April 3	Washington University, Eric P. Newman Education Center	St. Louis, MO
April 5 - 9	American Association of Cancer Research (AACR)	Toronto, Canada
April 11 - 15	Experimental Biology 2003 (EB)	San Diego, CA
April 24	University of Illinois, Illini Union	Chicago, IL
April 27 - May 1	8th International Society for the Study of Xenobiotics (ISSX)	Dijon, France
April	University of Georgia, Georgia Center	Athens, GA
End of April	2003 Apoptosis Conference	San Diego, CA
April 30 - May 3	California Blood Bank Society	Palm Springs, CA
May 1	Emory University, Cox Hall	Atlanta, GA
May 5 - 7	Conference on Vaccine Research	Arlington, VA
May 6 - 9	Cytomics Conference	Wales, UK
May 6 - 10	American Association of Immunologists (AAI)	Denver, CO
May 10 - 15	Environmental Mutagen Society (EMS) 2003	Miami Beach, FL
May 15	University of Utah, Jewish Community Center	Salt Lake City, UT
May 15 - 19	Federation of Clinical Immunology Societies (FOCIS)	Paris, France
May 20 - 22	American Society for Microbiology	Salt Lake City
May 22	15th Signal Transduction Symposium	Chicago, IL
June	Dartmouth Course	Hanover, NH
June 5	University of Colorado, Balch Field House	Boulder, CO
June 11	University of Pennsylvania, Houston Hall	Philadelphia, PA
June 25	University of Wisconsin, Union South	Madison, WI
June 22 - 25	BIO 2003 (International Biotechnology Convention and Exhibition)	Washington, D.C.
June 29 - July 3	Cytochromes P450	Prague, Czech Republic

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