# HotLines

VOL 6 NO 3 WINTER 2001




## IN THIS ISSUE:



# NEW PE-Cy7 and APC-Cy7 Tandem Conjugates for Flow Cytometric Analysis

By Kerstin Willmann, Tom Frey, Joe Link, Barny Abrams, and Ken Davis

### Increase Productivity and Expand Flexibility

Get more information out of a single tube than ever before with the NEW PE-Cy7* and APC-Cy7* tandem conjugates. Perform up to six-color analysis using FITC, PE*, PerCP* or PerCP-Cy5.5*, PE-Cy7, APC*, and APC-Cy7. The fifth and sixth fluorescence parameters add more flexibility to your experimental design in immunophenotyping, immune function studies, and cell sorting flow cytometry applications.

*Continued on page 2*

**BD Biosciences**
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BD

# NEW PE-Cy7 and APC-Cy7 Tandem Conjugates *(continued from cover)*

## Optimal Compensation and Bright Signals

Cy7 emits in the far-red region of the spectrum (767 nm) and is distinctly separated from other typically used fluorochromes in flow cytometry (Figure 1). For comparison, the spectral emission peak of PE is 565 nm, for PerCP 677 nm, PerCP-Cy5.5 695 nm, and for APC 660 nm. The chemistry of the new tandem conjugates is optimized for low compensation between the tandem conjugates and other fluorochromes (Figure 2).

## Instrumentation

PE-Cy7 is excited at 488 nm using a blue laser and can be run on single-laser instruments with the appropriate emission optics. APC-Cy7 is excited using a red laser such as a dye laser, a HeNe laser, or a Krypton laser. Instruments accommodating these needs are the FACS Vantage™, FACSVantage SE (standard analog or FACSDiVa), and the NEW custom LSR instrument, which will be available in Spring 2002. Instrument setup is performed manually or samples can be run uncompensated followed by digital compensation using the FACSDiVa Option (Figure 3) or FlowJo software.

## Superior Quality

Our reagents are manufactured and tested to high standards of purity, stability, low background, and lot-to-lot consistency. All fluorescent conjugates are optimized and ready for use in flow cytometry.

### Ordering Information for Anti-Human Products

Additional BD Biosciences Immunocytometry Systems antibodies can be conjugated to PE-Cy7 and APC-Cy7 through our custom conjugate program. Please contact your local BD Biosciences representative for more details.

| Product Description | | RUO[a] Cat. No. | ASR[b] Cat. No. |
|---|---|---|---|
| CD4 PE-Cy7 | *Coming soon!* | 348789 | 348799 |
| CD5 PE-Cy7 | *Coming soon!* | 348790 | 348800 |
| CD34 PE-Cy7 | *Coming soon!* | 348791 | 348801 |
| Mouse $IgG_1$ PE-Cy7 | *Coming soon!* | 348788 | 348798 |
| CD8 APC-Cy7 | *Coming soon!* | 348793 | 348803 |
| CD19 APC-Cy7 | *Coming soon!* | 348794 | 348804 |
| CD45 APC-Cy7 | *Coming soon!* | 348795 | 348805 |
| Mouse $IgG_1$ APC-Cy7 | *Coming soon!* | 348792 | 348802 |

[a] *For Research Use Only. Not for use in diagnostic or therapeutic procedures.*
[b] *Analyte Specific Reagent. Analytical and performance characteristics are not established. Available in the US only.*

### Ordering information for Anti-Mouse Products

| Product Description | Cat. No. |
|---|---|
| B220 APC-Cy7 | 0112FA |
| CD4 (GK1.5) APC-Cy7 | 552051 |

** Patent—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: US 4,542,104 APC-Cy7: US 5,714,386*

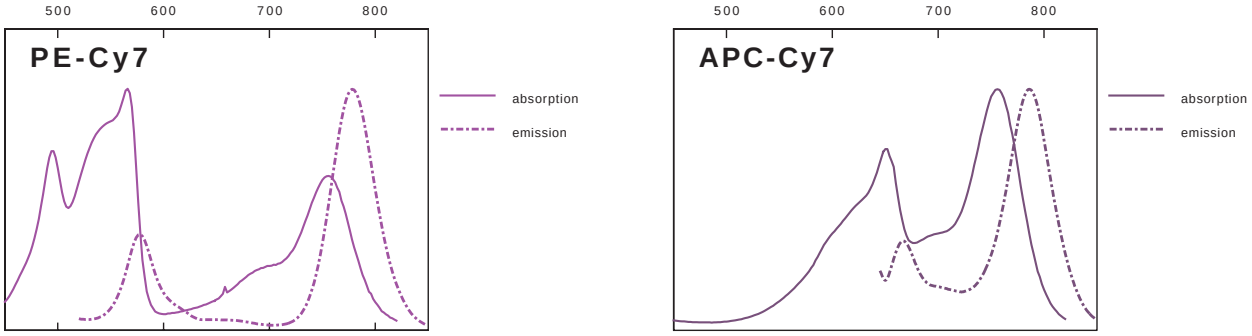



**Figure 1.** Absorption and emission spectra of PE-Cy7 and APC-Cy7 tandem fluorophores

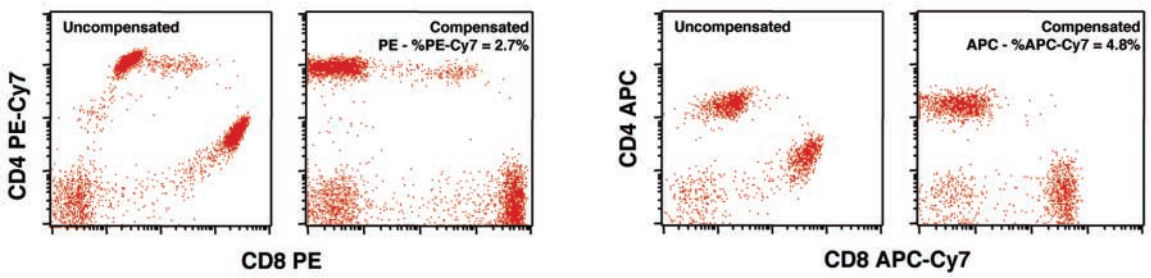
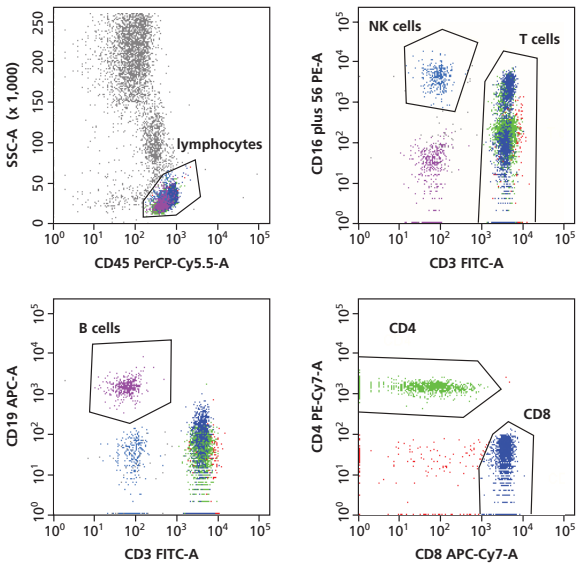



**Figure 2.** CD4 PE-Cy7 vs CD8 PE: compensated vs uncompensated data analyzed on an analog FACSVantage SE




Tube: 3/16+56/45/4/19/8/

| Population | #Events | %Parent | %Total |
|---|---|---|---|
| All Events | 10,000 | | 100.0 |
| Lymphocytes | 5,223 | 52.2 | 52.2 |
| T cells | 4,415 | 84.5 | 44.2 |
| CD8 | 1,988 | 45.0 | 19.9 |
| CD4 | 2,249 | 50.9 | 22.5 |
| NK cells | 322 | 6.2 | 3.2 |
| B cells | 394 | 7.5 | 3.9 |

**Figure 3.** Six-color fluorophore combination: CD3 FITC/CD16+56 PE/CD45 PerCP-Cy5.5/CD4 PE-Cy7/CD19 APC/CD8 APC-Cy7: Data were acquired and analyzed on a FACSVantage SE with the FACSDiVa Option digital electronics. All parameters are displayed on a five-decade scale using the area measurement. For more details on the FACSDiVa Option, please contact your local BD Biosciences representative.

# Identifying Antigen-Specific CD8+ T-Cell Responses

By Smita Ghanekar, Kerstin Willmann, and Holden T. Maecker

CD8+ T cells are the primary effector cells of acquired cellular immunity. They possess cytolytic activity against virally infected cells (or in some cases tumor cells) and can elaborate anti-viral cytokines[1]. In HIV disease, a powerful demonstration of the importance of CD8+ T cells was made in a macaque model of SIV[2]. Depletion of CD8+ T cells from macaques completely abolished their ability to be protected from disease. Evidence for the importance of CD8+ T cells in HIV also has come from studies of HIV– exposed, but uninfected, individuals who share strong CTL activity against particular epitopes of HIV[3-5]. These studies have raised interest in detecting and monitoring antigen-specific CD8+ T cells in HIV and cancer.


CD8+ T cells recognize peptide antigens bound to class I MHC molecules on host antigen-presenting cells. In order to detect CD8+ T-cell cytokine responses to complex antigens, it is necessary to present the relevant peptide epitopes of these antigens on host class I MHC molecules. Fortunately, this is easily accomplished by exogenous addition of peptides to whole blood or PBMCs[6]. In fact, a large number of peptides can be added as a mixture to a single tube or well. Studies by Kern and colleagues[7] have demonstrated the utility of using a mixture of overlapping 15 amino acid peptides to detect CD8+ T-cell responses to an entire protein. We, and others, have adapted this approach to a variety of protein antigens from CMV and HIV[8,9]. An advantage of using 15 amino acid peptides is that they are able to stimulate CD4+ T cells, while still stimulating CD8+ T cells with relative efficiency[9]. Using multiparameter cytokine flow cytometry (CFC), CD4+ and CD8+ functional responses can be easily resolved in a single assay.

Other methods for detecting antigen-specific CD8+ T cells include phenotypic analysis using recombinant, multivalent MHC-peptide complexes. The two most common constructs of this type are MHC-peptide tetramers[10] and MHC-Ig dimers[11] (DimerX reagents, BD Biosciences Pharmingen). These structures can be loaded with a specific recombinant peptide of choice and added to whole blood or PBMCs. Antigen-specific T cells can be detected by flow cytometry after MHC-peptide complexes bind to a specific T-cell subset. Both dimer and tetramer technologies enable interrogation of T cells of just one MHC-peptide specificity at a time. The response to a complex antigen such as HIV involves T-cell clonotypes specific for a number of different MHC-peptide combinations that are different in each individual[8,12]. It is important to note that dimer and tetramer technologies used in isolation do not provide a direct readout of a cell's functional capacity. These drawbacks are overcome by the use of functional assays, such as CFC or ELISPOT, that detect cytokine production by antigen-specific T cells and are suggested to correlate with cytotoxic activity[13]. By combining functional assays with tetramer or dimer analysis, functionally anergic cells of a particular specificity can be identified[14-16]. Anergic cells could contribute to the lack of immune responsiveness in chronic diseases like cancer. The dimer or tetramer approach is easily combined with functional CFC, since both techniques can be applied to the same sample and analyzed by flow cytometry. In contrast, ELISPOT technology uses an optical plate reader for sample analysis and is restricted to one- or two-parameter detection. Therefore, subsetting of functional and non-functional T-cell populations is easiest to address with CFC.

## Product Highlight

BD Biosciences Immunocytometry Systems has developed a step-by-step, highly reproducible methodology for performing CFC assays. Our FastImmune™ protocols are

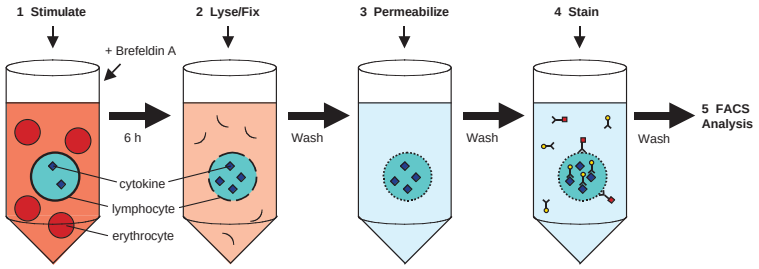



**Figure 1.** Overview of CFC methodology for whole blood

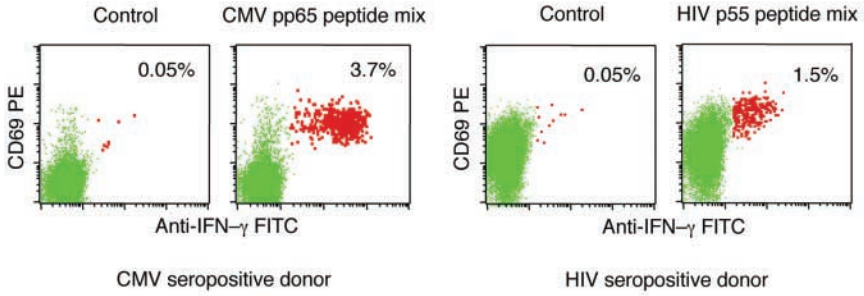



**Figure 2.** Detection of CMV– and HIV–specific $CD8^+$ T-cell responses using mixtures of overlapping 15 amino acid peptides spanning the CMV pp65 or HIV p55 glycoproteins: All plots are gated on $CD3^+CD8^+$ cells. For methods, see reference 9.

based on the use of individual antibody reagents, multi-color reagent products, or cytokine detection kits that include all reagents for sample preparation, thus providing assay standardization.

The NEW FastImmune CD8 Intracellular Cytokine Detection Kit directly identifies antigen-activated $CD8^+$ T-cell IFN-γ responses. The kit is developed for use with human whole blood or PBMCs. It includes a specific antibody combination, matching isotype control, and necessary sample processing reagents for optimal and highly reproducible results. The FastImmune methodology is compatible with the requirements of vaccine monitoring in clinical trials[17]. For example, whole blood samples can be activated and automatically cooled at the end of the six-hour activation period by means of a programmable water bath or similar device. Also, samples activated and fixed with FACS™ Lysing Solution* can be frozen at –80°C for shipment to a different site where processing and analysis are done in a batch process. Maecker et al found that cytokine responses to peptide antigens can be efficiently obtained with fresh and cryopreserved samples[9]. Streamlined procedures for analysis of $CD8^+$ T-cell responses in clinical trials have the potential to accelerate the identification of surrogate immunological endpoints in diseases such as HIV and cancer[18].

An overview of the CFC assay using whole blood is shown in Figure 1. Whole blood is subjected to antigenic stimulus in the presence of a secretion inhibitor, Brefeldin A (BFA). This leads to intracellular accumulation of newly synthesized protein. After a stimulation period of six hours, the leucocytes are fixed and erythrocytes lysed, simultaneously. The cells are then washed and permeabilized. Finally, intracellular, as well as surface, antigens are stained in the same protocol step with Anti-Hu IFN–γ FITC, CD69 PE, CD8 PerCP-Cy5.5, and CD3 APC, followed by flow cytometric analysis. The FastImmune CD8 Kit includes CD3 APC to avoid misidentification of NK cell responses (CD8dim) upon antigenic stimulus. $CD8^+$ T cells are identified as $CD3^+CD8^+$ events, and their functional responses are measured as cells positive for both CD69 PE and Anti-IFN–γ FITC. The use of CD69, an early activation marker, provides more confidence in

# Identifying Antigen-Specific CD8+ T-Cell Responses *(continued from page 5)*

**Table 1. Typical CD8+ T-cell response ranges for various stimuli in healthy donors (estimated by BD Biosciences Research and Development group).**

| Stimulus | Typical %CD3+CD8+CD69+IFN-γ+cells |
|---|---|
| PMA+ionomycin | 70–90 |
| Staphylococcal enterotoxin B (SEB) | 1–15 |
| Specific antigen (eg, CMV pp65 peptide mix) | 0.2–5 |

identifying cytokine-positive cells as having been recently activated, either *in vivo*, or as a result of the *in vitro* stimulation. CD69 staining also permits better visual clustering of cytokine-positive cells in a CD69 vs Anti-IFN–γ dot plot. This can aid in the identification of rare populations of antigen-specific cells. A typical example of CD8+ T-cell responses using a peptide-mix antigen is shown in Figure 2.

In intracellular staining procedures, the requirements for optimal staining intensity are very stringent. BD Biosciences has selected monoclonal antibody clones and optimized conjugation chemistry to achieve bright signals and minimal background. Table 1 provides a general reference of typical ranges of cytokine responses observed using various stimuli. While individual results may vary, antigen-specific CFC assays demand the use of reagents and techniques that provide very low background in order to detect responses of this magnitude.

**References**

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## NEW Specific Antigen-Activation Intracellular Cytokine Detection Kits

FastImmune Cytokine Detection Kits make getting started easier with a simplified protocol, optimized reagents, and an open system for the activation antigen of your choice. Each kit provides sufficient reagents to stain 25 stimulated (specific and isotype control tube) and 25 unstimulated (specific and isotype control tube) human whole blood samples.

### Kits contains:

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

| Cat. No. | | Product Description† | |
|---|---|---|---|
| 346049 | **NEW** | FastImmune CD8 Anti-Hu IFN-γ Detection Kit | *Coming soon* |
| 340970 | | FastImmune CD4 Anti-Hu IFN-γ Detection Kit | |
| 340971 | | FastImmune CD4 Anti-Hu IL-2 Detection Kit | |
| 340972 | | FastImmune CD4 Anti-Hu TNF-α Detection Kit | |

## NEW Specific Multicolor Cytokine Reagents

FastImmune three- and four-color cytokine reagents can be used alone or in conjunction with the Intracellular Cytokine Detection Kit for additional cytokine measurements. These reagents have already been optimized to work together, so you don't have to perform tedious titrations or additional pipeting steps during your assay. CD4 and CD8 are conjugated to PerCP-Cy5.5 for better separation of CD4dim and CD8 dim T cells from the negative cell population.

| Cat. No. | | Product Description† | |
|---|---|---|---|
| 346048 | **NEW** | FastImmune Anti-Hu IFN-γ FITC/CD69 PE/CD8 PerCP-Cy5.5/CD3 APC | *Coming soon* |
| 346047 | **NEW** | FastImmune $IgG_{2a}$ FITC/$IgG_1$ PE /CD8 PerCP-Cy5.5/CD3 APC | *Coming soon* |
| 340962 | | FastImmune Anti-Hu IFN-γ FITC/CD69 PE/CD4 PerCP-Cy5.5 | |
| 340963 | | FastImmune Anti-Hu IL-2 FITC/CD69 PE/CD4 PerCP-Cy5.5 | |
| 340964 | | FastImmune Anti-Hu TNF-α FITC/CD69 PE/CD4 PerCP-Cy5.5 | |
| 340965 | | FastImmune $IgG_{2a}$ FITC/$IgG_1$ PE/CD4 PerCP-Cy5.5 | |

*For Research Use Only. Not for use in diagnostic or therapeutic procedures. Not for resale.*

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

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

# Standardization of the Human Th1/Th2 Cytometric Bead Array (CBA) Cytokine Standards to International Standards

By Homero Sepulveda, Ph.D., Heather Baumhover, Diane Mochizuki, Ph.D., Jerry Wilson, and David Ernst, Ph.D.

Laboratories throughout the world use different bioassays and immunoassays to measure and report cytokine protein levels that are present in biological samples. For this reason, the availability of international standard preparations of cytokine proteins is essential to allow definitive analyses and comparison of results[1]. These primary (*aka*, gold) standards are frequently used to calibrate biological activities and protein concentrations between different secondary assay standards used by investigators. The gold standards provide a means to determine relative concentrations of unknown samples and an ability to compare results between experiments and laboratories. In order to support the comparison of cytokine protein measurements obtained using the Human Th1/Th2 Cytokine CBA Kits (Table 2), we evaluated the assay performance of the standards provided in the CBA Kit with gold standards from the National Institute for Biological Standards and Control (NIBSC).

The NIBSC Human Cytokine Protein Standards are recognized by the World Health Organization (WHO) as International Biological Standards. They meet established requirements for accuracy, consistency and stability[2]. The NIBSC/WHO standards are assigned potency values in International Units (IU) of biological activity and nominal mass (*i.e.*, not absolute mass values) for purposes of bioassay and immunoassay determinations[1]. These International Standards are not intended to be used as samples of purified material. Consequently, they cannot be used to establish absolute concentrations or specific activities for cytokine preparations. Rather, the standards provide a means to facilitate comparisons of cytokine concentration values determined by experiments conducted within the same or different laboratories.

Herein, we compared the performance and expected concentration of the International Cytokine Standards relative to our Human Th1/Th2 CBA Cytokine Standards. The resulting data, together with the conversion factors between the CBA standards and the International Standards (*i.e.*, nominal mass values) are summarized in Table 1. As shown in Figure 1, the performance of both sets of standards was found to be similar as measured by observed parallelism of the dose response slope. This observed parallelism provided confidence that comparisons of the concentrations of the two standards (and subsequent quantitation of native biological samples) are valid. It is important to note that the standard's source (*i.e.*, insect cell, *E. coli*, etc.) can greatly effect the measurement and performance of a protein in a given antibody-based immunoassay. The conversion factors for the NIBSC/WHO standards make it possible to determine the equivalency of cytokine protein concentrations present in samples measured by different immunoassays that have been standardized to the same NIBSC/WHO standards.

**References:**

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2. Biological Reference Materials 2000. National Institute for Biological Standards and Control.

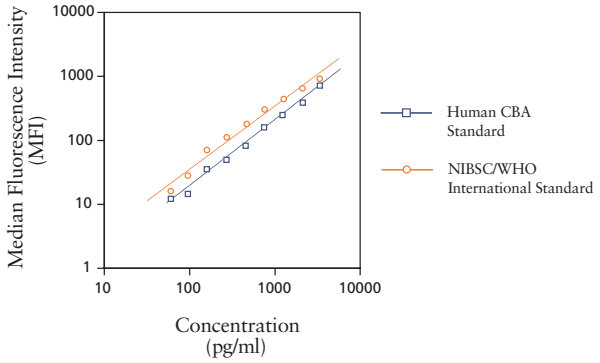



**Figure 1. Human IFN-γ titration curves.**

### Table 1. Conversion Factors Between the BD CBA Human Th1/Th2 Cytokine Standards and the NIBSC Standards.

| | Human IL-2 | Human IL-4 | Human IL-5 | Human IL-6 | Human IL-10 | Human TNF-α | Human IFN-γ |
|---|---|---|---|---|---|---|---|
| NIBSC Code Number | 86/504 | 88/656 | 90/586 | 89/548 | 93/722 | 87/650 | 87/586 |
| I.U. | 100 I.U. | 1000 I.U. | 5000 I.U. | 100,000 I.U. | 5000 I.U. | 40,000 I.U. | 250 I.U. |
| Mass units per vial | 7.6 ng | 100 ng | 500 ng | 1000 ng | 1000 ng | 1000 ng | 12.5 ng |
| Nominal NIBSC concentration (pg/ml) | 5000 | 5000 | 5000 | 5000 | 5000 | 5000 | 5000 |
| Calculated concentration using CBA (pg/ml) | 3296 ± 635 | 4465 ± 479 | 7811 ± 639 | 5813 ± 755 | 4443 ± 671 | 6568 ± 327 | 10848 ± 599 |
| CBA:NIBSC/WHO Mass Conversion Factor | 1.52 | 1.12 | 0.64 | 0.86 | 1.13 | 0.76 | 0.46 |

*Table 1. The relationships of the CBA and NIBSC cytokine standards were determined in a parallel titration study. In each case, the NIBSC standard was titrated (starting at 5000 pg/ml) based on its reported nominal mass/vial and tested with the CBA standards using the CBA assay. The calculated concentrations (mean ± SEM; n = 3) for the NIBSC standards were used to determine conversion factors for standardizing sample concentrations determined with the CBA standards.*

### Table 2. BD CBA Products available from BD Biosciences Pharmingen

| Cat. No. | Description | Analytes | Format | Size |
|---|---|---|---|---|
| 552124 | Human Active Caspase-3 CBA Kit | Active Caspase-3 | Kit | 100 tests |
| 550749 | Human Th1/Th2 Cytokine CBA Kit | IL-2, IL-4, IL-5, IL-10, TNF-α, IFN-γ | Kit | 50 tests |
| 551809 | Human Th1/Th2 Cytokine CBA Kit - II | IL-2, IL-4, IL-6, IL-10, TNF-α, IFN-γ | Kit | 50 tests |
| 551811 | Human Inflammation CBA Kit | IL-8, IL-1β, IL-6, IL-10, TNF-α, IL-12p70 | Kit | 50 tests |
| 551810 | Human Th1/Th2 Cytokine Standards | IL-2, IL-4, IL-5, IL-6, IL-10, TNF-α, IFN-γ | lyophilized | 1 vial |
| please inquire | Human Anaphylatoxin CBA Kit | C3a, C4a, C5a | Kit | 50 tests |
| 551287 | Mouse Th1/Th2 Cytokine CBA Kit | IL-2, IL-4, IL-5, TNF-α, IFN-γ | Kit | 50 tests |
| 550026 | Mouse Immunoglobulin Isotyping CBA Kit | Heavy and light chain isotypes of mouse IgG1, IgG2a, IgG2b, IgG3, IgA, IgM, IgE | Kit | 100 tests |
| 550065 | BD CBA Software | Mac and PC Compatible CD-Rom and User's guide | CD | 1 CD |

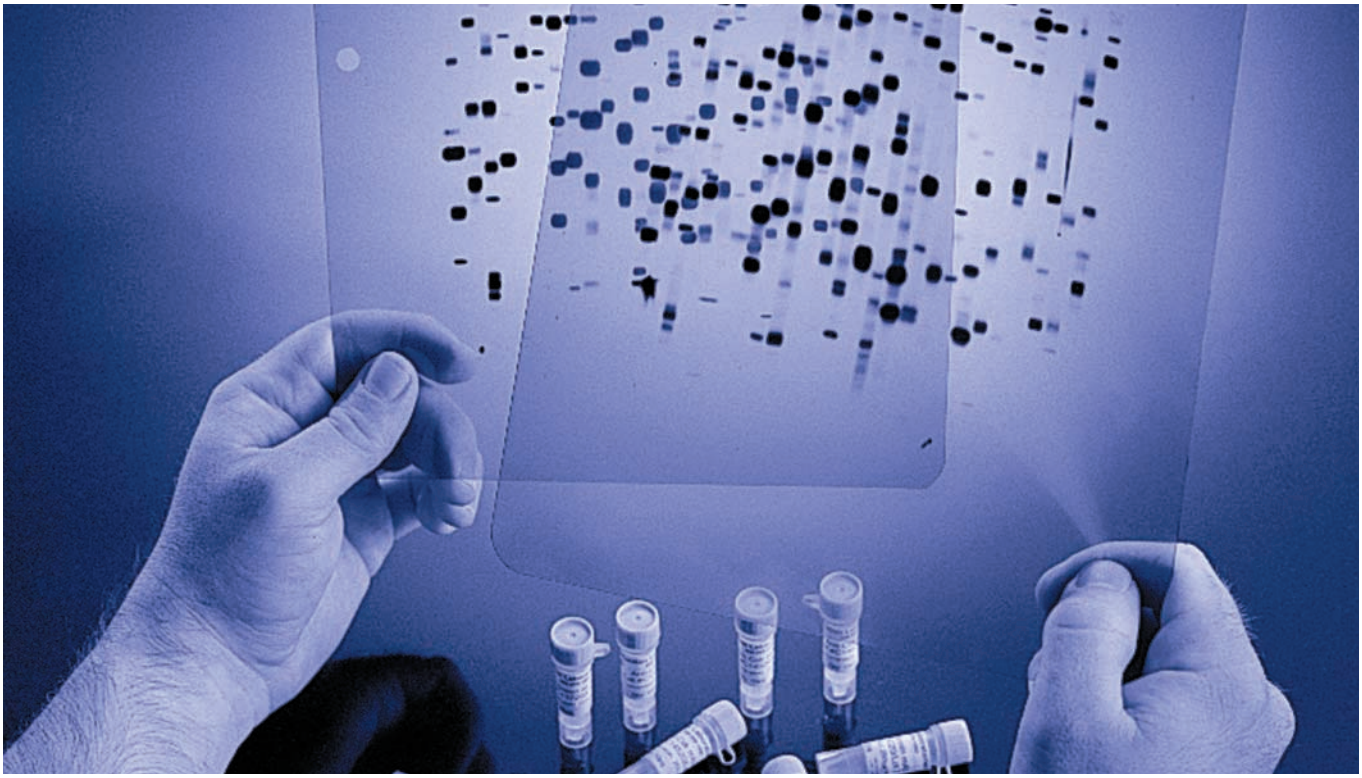
## Announcing the newest addition to the BD Cytometric Bead Array (CBA) Product Portfolio - Human Active Caspase-3 CBA Kit

BD Biosciences is pleased to announce the release of the Human Active Caspase-3 CBA Kit. The Human Active Caspase-3 CBA Kit is a single bead assay for the rapid quantitative measurement of active caspase-3 protein from small volume cell lysate samples. The assay is capable of measuring active caspase-3 protein over a two-log dynamic range using a simple 4-hour assay protocol, providing a significant improvement over conventional western blot assays. Please contact BD Biosciences Pharmingen Technical Services for more details.

Catalog Number 552124

*To see more of this image and read about the "Multiplex Bead System You Can Count On," please see the end of this issue.*

# News Update from the BD Biosciences Custom Products & Services Group



By Nicole Johnson, Jay Dong, and Tony Ward

With the integration of several businesses across BD Biosciences, we have created a single custom products and services group (or CPSG for short) to better serve you, our valued customer. This group combines the Custom products and services from the Custom Technology Team (BD Biosciences Pharmingen), molecular products and services (BD Biosciences Clontech), ADME/Tox and custom plasticware (BD Biosciences Discovery Labware) and custom flow cytometry related instrumentation (BD Biosciences Immunocytometry Systems). Together, we offer an unparalleled range of custom products and services to streamline and optimize your work. Listed below are some of our latest offerings.

## Custom Products

Custom products are a convenient solution to answer your unmet needs.

### Multicolor flow cytometric cocktails with new tandem conjugates

Explore multicolor flow cytometry with 4- and 5-color antibody cocktails utilizing new dyes such as PerCP-Cy5.5 and PE-Cy7.

### Cytokine Flow Cytometry Products

Measure antigen-specific immune response in whole blood activated with your antigen or peptide pools. Detect both Th1 and Th2 cytokines intracellularly along with surface cell markers. These products are available for both Human and Non-Human Primate studies.

### Cytometric Bead Array (CBA) testing service

Multiplex detection of multiple analytes in small serum and plasma samples (choose from Th1/Th2 and inflammatory cytokines, and apoptosis markers).

### Cell proliferation with BrdU by flow cytometry

Proliferation assays using antibodies tracking incorporation of BrdU in combination with cell surface markers. Unlike bulk 3H-thymidine incorporation assays, these assays let you know exactly which cells are proliferating in response to your antigen.

### Made to order Multiple Tissue Arrays (MTA)

Need to verify or localize your genes and proteins? You provide the tissues, specify the punch size and any other parameters, and we will make your MTA for you. We can also help you pre-screen or validate your data for your particular gene or protein using our on-site pathologist.

## Custom Services

In general, if we offer a technology as a custom product, we also can provide a contract service too. In addition, we offer the following dedicated services:

### ELISPOT testing services

Measure frequency of cytokine-producing cells. Assays are optimized for detecting Th1 (IL-2, IFN-γ, TNF-α) and Th2 (IL-4, IL-5) cytokine secretion by T cells.

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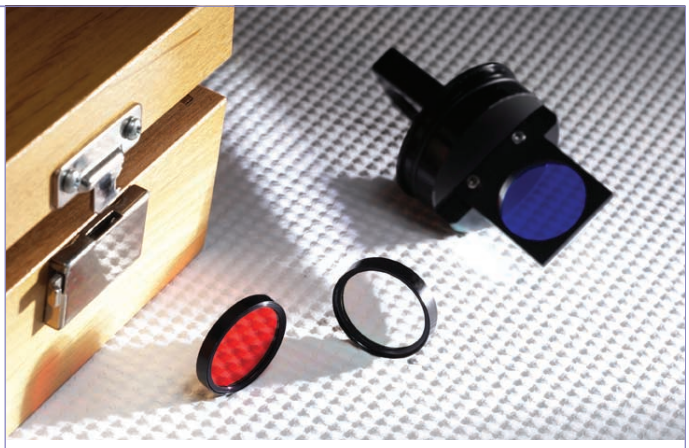
# Identification of a Primitive Progenitor Cell Subset Using Hoechst 33342

By Dennis T. Sasaki, B.Sc., MA



## Introduction

Characterization of hematopoietic stem cells and their subsets has traditionally been performed with cell surface staining using fluorescently labelled monoclonal antibodies. A cell marker of choice is CD34[1], a transmembrane phosphoglycoprotein that is selectively expressed on vascular endothelial cells, tissue fibroblasts or stromal cells, and hematopoietic cells. CD34 has been used in different combinations with other cell surface markers and is the most prevalent marker currently used for identification of mouse and human progenitor cells. The cells identified by these methods have biological properties that include self renewal and differentiation into mature cell lineages[2,3].

Fluorescent probes such as rhodamine 123 [4,5] and pyronin Y[6] have been shown to fractionate subpopulations of hematopoietic progenitor cells when used with other cell surface markers. Goodell[7,8,9] and co-workers have described a method using dual wavelength flow cytometry to define a small (0.01-0.10%) population of primitive progenitor cells that selectively efflux the dye Hoechst 33342 (bis-benzimide) in mouse, primate, and human bone marrow as well as in a variety of tissues. This population of cells is termed "side population" (SP) cells. These cells are enumerated by exciting the Hoechst labelled cells with the 350 nm line of a UV laser and then simultaneously measuring the fluorescence using blue and far red emission filters. Of particular interest is that these cells express CD34-low to negative and are thought to be the most primative of progentitor cells identified to date.

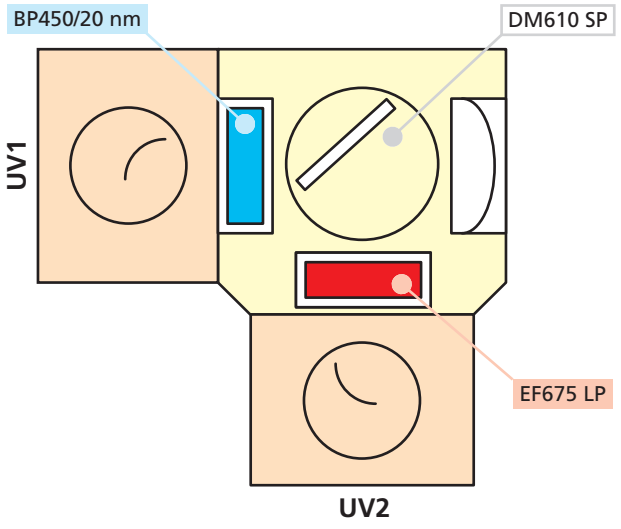



**Figure 1.** Optical diagram showing the position of the filter set that includes the following filters: DM610 SP, BP450/20, and EF675 LP.

## Method

The SP hematopoietic stem cells are identified by the cell's ability to differentially efflux the Hoechst dye via a multi-drug-like transport mechanism. This is a dynamic process, therefore care must be exercised during the labeling protocol by judiciously observing the time and temperature requirements[10].

The **BD Biosciences Stem Cell Side-Population Filter Set, (Cat. No. 341063)**, provides operators of the BD FACStarPlus™, BD FACSVantage™, BD FACSVantage™ SE (with or without the FACSDiVa option) with a convenient way to set up flow cytometers equipped with an ultraviolet laser in the second or third positions to perform these experiments. The DM610 SP used to distinguish the red from the blue fluorescence signals comes premounted on a mirror holder that is attached to the instrument's dichroic mirror holder. The BP450/20 nm and EF675-LP filters are then installed in front of the PMT detectors as shown in Figure 1.

The BP450/20 nm is used to discriminate the fluorescent blue signal and the EF675-LP the red signal. The events are collected using linear amplification (Figure 2).

## For best results:

1. UV laser should be tuned to 350 nm and 100 mW for defining this rare sub-population of cells.
2. Data should be displayed as correlated Hoechst blue and Hoechst red in linear mode.
3. Cytometer should be aligned to minimize the cv's in all of the fluorescence channels for this application.
4. The sample should be kept at 4°C throughout the analysis or sort to minimize dye efflux.
5. Sorted S-P cells should be collected at 4°C in a small volume of DMEM containing 10% FBS and 10 mM HEPES (use a 1.5 ml Eppendorf centrifuge tube).
6. Maintain a low sample differential for maximum resolution.
7. Collect a large list mode data file of 100,000 events for the analysis.
8. Gate out the dead cells using propidium iodide (PI).

The phenomenon of 'red fluorescence' from Hoechst 33342 is not fully understood. It is postulated that the way in which the dye binds to the chromatin conformation has a direct effect on the emission spectra[11].

BD FACSVantage SE

## Results:

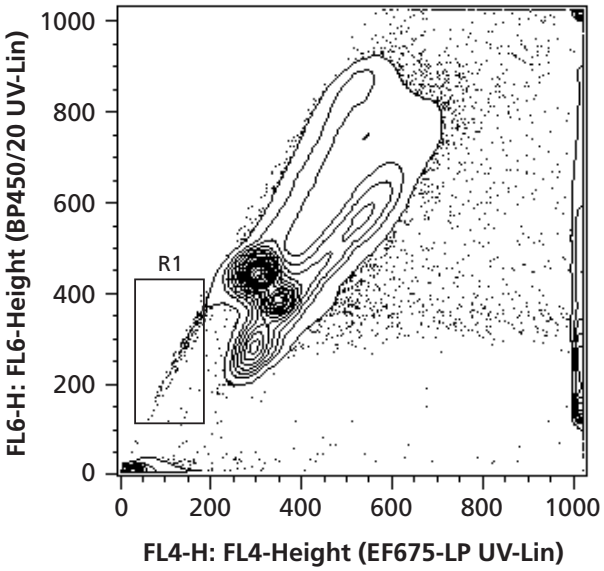



**Figure 2.** Murine C57BL/6 bone marrow was stained using Hoechst 33342. A data set of 100,000 events was collected in list mode. The contour plot at 5% probability of ungated events show the S-P population (R1) with frequencies that range from 0.02 to 0.08%.

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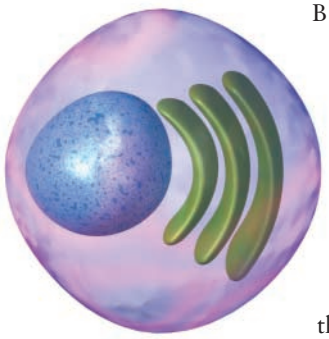
# Developing B lymphocytes in the Mouse

By Michelle Krakowski, Andrea Nguyen, and Belen Ybarrondo

Mouse B lymphocytes are generated from pluripotent hematopoietic stem cells found in the liver before birth and in the bone marrow afterward. Much effort has been made to understand the stepwise and highly regulated process of B cell development, or B lymphopoiesis. Of course, this process is extremely complex, and the survival of developing B cells is tightly linked to requirements for various growth factors and the appropriate temporal expression of signaling molecules promoting differentiation, proliferation and viability. The expression or loss of specific molecules found either on the cell surface or in the cytoplasm, has been used as a key to interpret the differentiation stage of a given developing B cell. Various independent but correlated nomenclatures have been created to define the steps from stem cell to mature B cell, and we have attempted to represent a unified model (Figure 1). This discussion will focus on the B cells originating from the bone marrow. For a more detailed description of liver-derived B cells and B1 cells, please see references 3, 4, and 7.



Very early B lymphocyte progenitors are found within a pool of CD117/c-kit$^+$ CD127/IL-7R$\alpha^+$ common progenitor cells that have the capacity to develop along not only B but also T and NK lineages. Among an ever-increasing list of transcription factors (TFs) involved in B cell development, E2A and EBF clearly initiate B cell-specific gene expression, including activation of Pax5, which then acts as the critical factor determining commitment to the B cell lineage[11,2,13]. This earliest committed developing B cell (a pre-pro-B cell) expresses low levels of the recombination activating gene products RAG1 and RAG2 as well as TdT (terminal deoxynucleotidyl transferase) otherwise known as the "rearrangement machinery". Thus these cells have not yet initiated immunoglobulin (Ig) gene rearrangement.

The next goal is to produce a functional mature B cell with a non-autoreactive immunoglobulin[9]. Pro-B/pre-B I cells are phenotypically defined as CD45R/B220$^+$ CD19$^{+/-}$ CD117/c–kit$^+$ CD43$^+$ CD25$^-$ IgM$^-$ IgD$^-$, but the critical aspect that sets these cells apart is the initiation of $D_H$ (diversity) to $J_H$ (joining) segment rearrangement.

The transition to the pre-B/pre-B II cell requires $V_H$ (variable) segment rearrangement with the previously made $DJ_H$. The randomness of segment recombination is critical for the great diversity of the Ig repertoire, but it also creates an extremely large number of out-of-frame, non-coding µH chains. Indeed, it is at this next stage of B cell development that the greatest numbers of apoptotic cells are found[5] as well as the greatest sensitivity to the growth and proliferation factor IL-7[15].

B lymphocyte development to the large pre-B/large pre-B II cell is critically dependent upon the ability of the newly made µH chain to associate with the surrogate light (SL) chain. This surrogate for conventional light chain is composed of the VpreB and λ5 proteins and is absolutely required for proper folding and membrane transport of Igµ during lymphopoiesis. Only those µH chains that pair with a SL chain to form the pre-B cell receptor (pre-BCR) along with the signaling proteins Ig$\alpha$ (CD79a) and Ig$\beta$ (CD79b) are allowed to continue developing[10]. The monoclonal antibody (mAb) SL156 may be used for the identification of such an appropriately complexed pre-BCR (Figure 2), as it specifically binds a conformational epitope of SL/µH complex on a subpopulation of pre-B/pre-B II cells[16]. The fully assembled pre-BCR triggers a proliferative expansion in these large pre-B/large pre-B II cells. Current studies have not yet determined whether surface expression of the pre-BCR, or some as yet unknown pre-BCR ligand cross-linking is required for this to occur. Also at this stage, expression of both the rearrangement machinery and SL chain are down-regulated, functionally inducing allelic exclusion for the heavy chain.

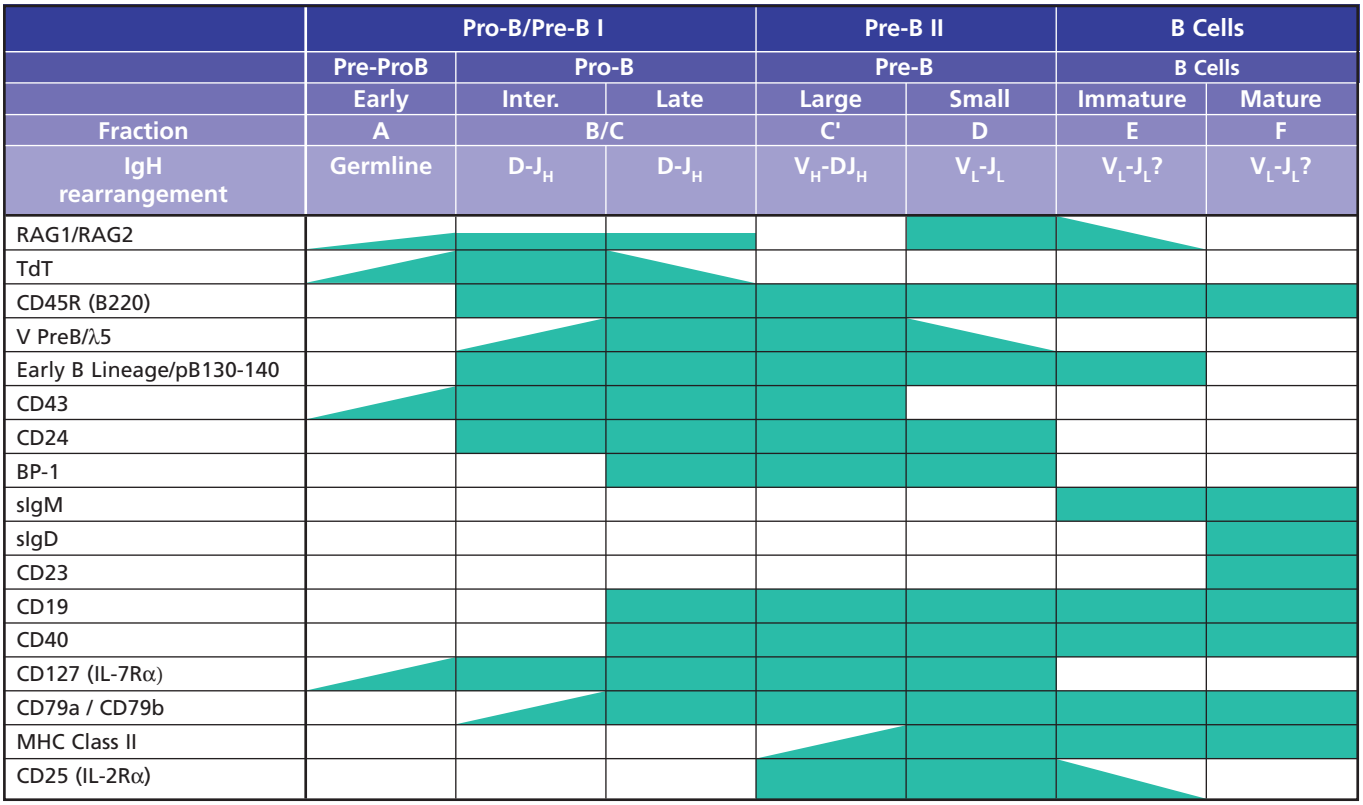



**Figure 1.** Genetic and phenotypic characterization of B cell maturation in mouse bone marrow.

After a limited number of cell divisions, pre-B/pre-B II cells fall into a resting state and are characterized by their small size (small pre-B/small pre-B II). Rearrangement of the light chain gene segments (κ first, and λ when necessary) is initiated, and successful production of an IgL is tested. It is now the ability of the IgL chain to productively pair with the pre-existing μH chain in cells to form a B cell receptor (BCR) that determines fitness. Additionally, cells with auto-reactive specificities are deleted or anergized unless they are able to further rearrange or "edit" their receptor.

An immature B cell is one able to express a non-auto-reactive BCR molecule and is phenotypically characterized as CD45R/B220$^+$ CD19$^+$ CD43$^-$ CD117/c–kit$^-$ CD25$^-$ IgM$^+$ IgD$^-$. Of the 2 x $10^7$ immature B cells produced by the bone marrow daily, approximately 10% reach the spleen, with even fewer making it into the re-circulating repertoire of mature B cells[1,5].

The transition from a developing B to a mature B cell may be identified using a combination of monoclonal antibodies specific for CD45R (clone RA3-6B2) and pB130-140 (clone 493), as seen in Figure 3[13]. Loss of expression of pB130-140 correlates well with emerging IgM and IgD expression as expected. Even within the periphery, selective pressures on the fitness of the BCR may come into play within the germinal center and secondary Ig gene rearrangement.

A developing B cell is required to make a functional heavy chain, assemble pre-BCR, pair heavy and light chains and delete auto-reactivity; overall a rare event. The stepwise progression of precursors through B lymphopoiesis is a tightly regulated system dependent upon survival signals balanced with apoptosis. The next challenges are to further our understanding of both the molecular mechanisms controlling these processes as well as the ability of the system to respond to the selective pressures shaping the overall peripheral repertoire.

# Developing B lymphocytes in the Mouse *(continued from page 15)*

**Table 1. Clones available from BD Biosciences Pharmingen for Mouse B Cell Development Research.**

| | Specificity | Clones |
|---|---|---|
| | CD5 | 53-7.3 |
| | CD16/32 (FcγR) | 2.4G2 |
| | CD19 | 1D3 |
| | CD24 (HSA) | 30-F1, J11D, M1/69 |
| | CD25 (IL-2Rα) | PC61, 7D4, 3C7 |
| | CD34 | RAM34 |
| | CD35 (CR1, CD21b) | 8C12 |
| | CD40 | HM40-3, 3/23 |
| | CD43 (Ly-48, Leukosialin) | S7 |
| | CD45, CD45.1, CD45.2 (Leukocyte Common Antigen, Ly-5) | 30-F11, A20, 104 |
| | CD45R (B220) | RA3-6B2 |
| | CD72 (Lyb-2.1) | 10-1.D.2 |
| | CD79a (Igα) | HM47 |
| | CD79b (Igβ) | HM79b |
| | CD80 (B7-1) | 1G10, 10-10A1 |
| | CD81 (TAPA1) | Eat1 |
| | CD86 (B7-2) | GL1, PO3 |
| | CD95 (fas) | Jo2 |
| | CD117 (c-kit) | 2B8, ACK45 |
| | CD127 (IL-7Rα) | B12-1 |
| | CD135 (Ly-72, Flk-2/Flk-3) | A2F10.1 |
| **NEW** | pB130-140 | 493 |
| **NEW** | Early B lineage | AA4.1 |
| | Flk-1 (VEGF-R2) | Avas 12a1 |
| | IgD | 11-26c.2a |
| | IgM | 11/41, R6-60.2 |
| **NEW** | λ5 | LM34 |
| | Ly-51 (BP-1 Antigen) | 6C3 |
| | OX40L | RM134L |
| | Rag-1 | G189-1417, G109-256 |
| | Rag-2 | G176-142, G110-461 |
| | TdT (Terminal deoxynucleotidyl transferase) | E17-1519 |
| **NEW** | VpreB | SL156 |

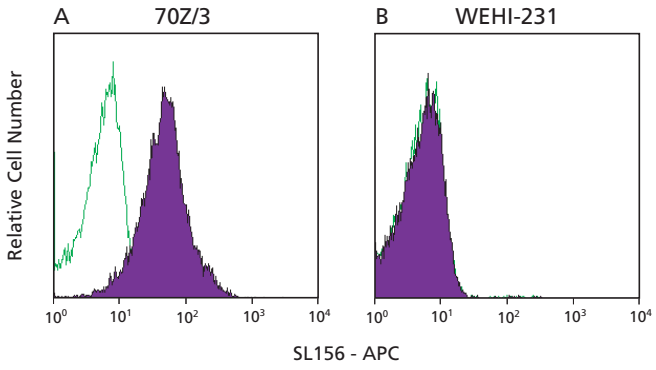



**Figure 2.** Pre-BCR is expressed on B cell lines resembling early stages of B cell development (70Z/3 a $sIg^-$ pre-B lymphoblast, Panel A), but not on those with a mature B cell phenotype (WEHI-231 a $sIgM^+$ B lymphocyte, Panel B). Cell lines were stained with the purified mAb SL156 (solid histogram) or isotype control rat $IgG_{2a}$ κ mAb R35-95 (open histogram), followed by biotinylated anti-rat $IgG_{2a}$ mAb RG7/1.30 then Streptavidin-APC. Dead cells were initially removed from analysis by propidium iodide gating. Flow cytometry was performed on a FACSCalibur flow cytometer (BD Biosciences, San Jose, CA).

*Unpublished BD Biosciences Pharmingen data.*

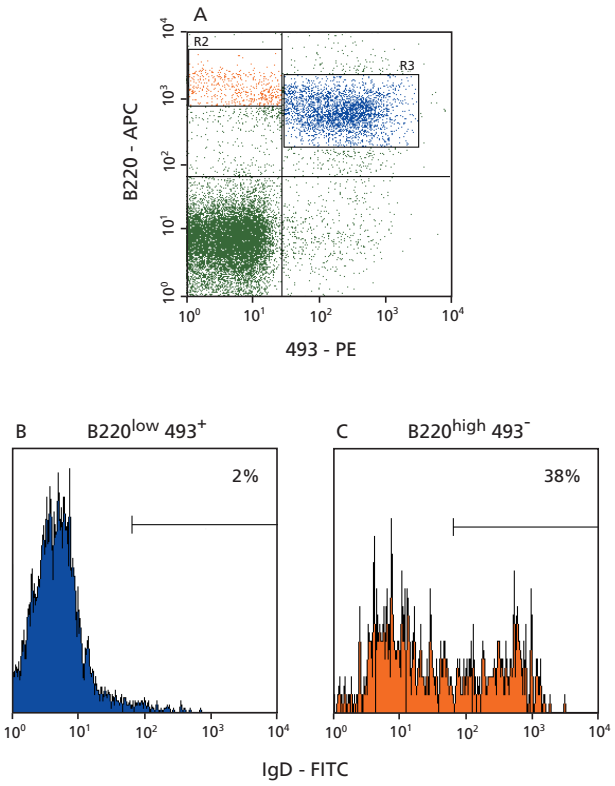



**Figure 3.** Transition from developing B cell to mature B cell. Bone marrow leukocytes from a 6 week old female BALB/c mouse were stained with APC-conjugated anti-mouse CD45R/B220 (RA3-6B2), PE-conjugated anti-mouse pB130-140 (493) and FITC-conjugated anti-mouse IgD (11-26c.2a). For analysis, gates were set to $B220^{low}$ $493^{+}$ developing B cells or $B220^{high}$, $493^{-}$ mature B cells (Panel A). As expected, no IgD expression was found on the developing B cells (Panel B), but was present on the more mature B cells (Panel C). Dead cells were initially removed from analysis by propidium iodide gating. Flow cytometry was performed on a FACSCalibur flow cytometer (BD Biosciences, San Jose, CA).

*Unpublished BD Biosciences Pharmingen data.*

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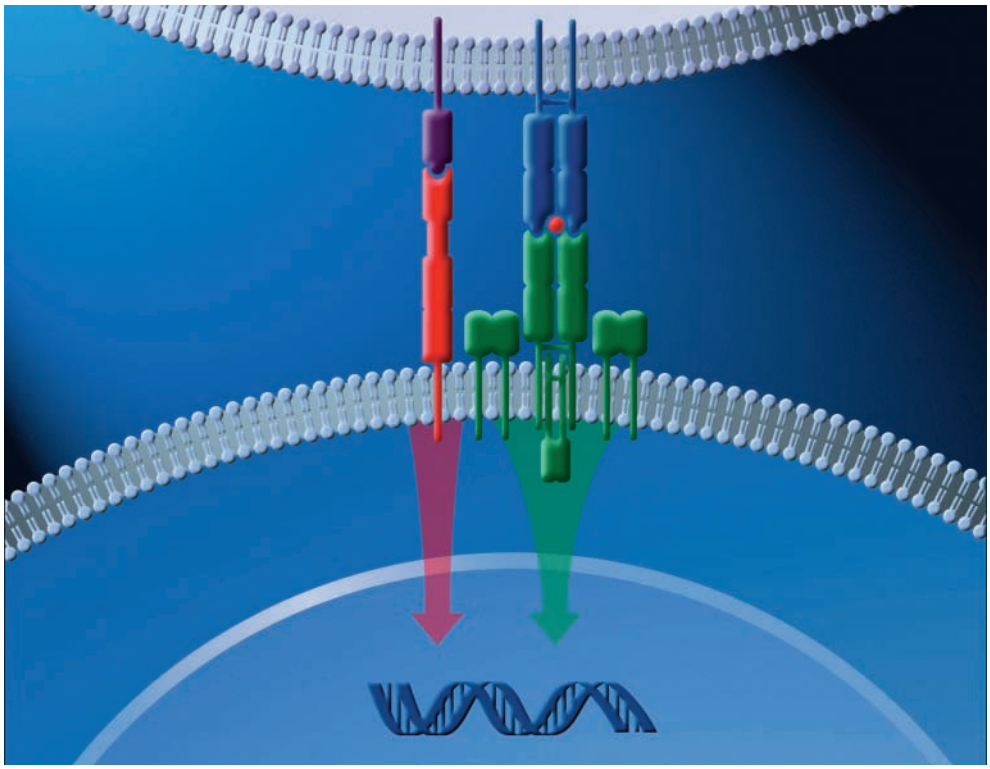
# NEW Reagents for the Study of Mouse T-Cell Costimulation



BD Pharmingen™ Purified anti-mouse ICOS mAb 7E.17G9, Purifed and PE-conjugated anti-mouse PD-1 mAb J43, and NA/LE™ Non-Cytolytic Mouse CTLA-4-IgG Fusion Protein are the latest additions to our line of research reagents for the expanding CD28 family of costimulatory receptors. The 7E.17G9 and J43 antibodies are useful for flow cytometric analysis of cell-surface expression of the ICOS and PD-1 antigens, respectively, on activated mouse leukocytes. The NA/LE™ Non-Cytolytic Mouse CTLA-4-IgG Fusion Protein blocks binding of both CTLA-4 and CD28 to their ligands, B7-1 and B7-2. For more detailed information about these reagents, please refer to the Technical Documents in our *e*Catalog at **www.bdbiosciences.com**.

The receptors of the CD28 family are cell-surface glycoproteins of the Ig superfamily which provide regulatory signals to either promote or inhibit T-Cell Receptor (TCR)–mediated activation of T lymphocytes. Their ligands, members of the B7 family, are expressed on antigen-presenting cells. Interactions of the CD28 and B7 families are involved in the regulation of T-cell activation and the establishment and maintenance of immunological tolerance.

**References**

Chambers C.A. The expanding world of co-stimulation: the two-signal model revisited. Trends in Immunology. 2001; 22:217-223.

Latchman Y, Wood CR, Chernova T, et al. PD-L2 is a second ligand for PD-1 and inhibits T cell activation. Nature Immunology. 2001; 2:261-268.

Table 1. Summary of the CD28 Family Members

| Receptor (Synonym) | Ligand (Synonym) | Regulatory Activity | Receptor Expression on Mouse Leukocytes |
|---|---|---|---|
| CD28 | CD80 (B7-1, BB1), CD86 (B7-2, B70) | Stimulation | Thymocytes; T, NK, and mast cells |
| CD152 (CTLA-4) | CD80 (B7-1, BB1), CD86 (B7-2, B70) | Inhibition | Activated T cells |
| ICOS | B7RP-1 (B7h, LICOS, B7-H2) | Stimulation | Activated T cells |
| PD-1 | PD-L1 (B7-H1), PD-L2 | Inhibition | Activated T, B, and myeloid cells |

## Table 2. BD Biosciences Reagents for T-Cell Costimulation Research

| Cat. No. | Description | Clone Name | Format | Size |
|---|---|---|---|---|
| **Reagents for TCR-mediated activation of mouse T lymphocytes** | | | | |
| 553057 | anti-mouse CD3e (CD3 ε chain) | 145-2C11 | NA/LE | 0.5 mg |
| 557306 | anti-mouse CD3e (CD3 ε chain) | 145-2C11 | Purified | 0.1 mg |
| 553058 | anti-mouse CD3e (CD3 ε chain) | 145-2C11 | Purified | 0.5 mg |
| 553238 | anti-mouse CD3e (CD3 ε chain) | 500A2 | Purified | 0.5 mg |
| 555273 | anti-mouse CD3 molecular complex | 17A2 | Purified | 0.5 mg |
| 553180 | anti-mouse γ δ TCR* | GL4 | Purified | 0.5 mg |
| 553181 | anti-mouse γ δ TCR* | UC7-13D5 | NA/LE | 0.5 mg |
| 553182 | anti-mouse γ δ TCR* | UC7-13D5 | Purified | 0.5 mg |
| 553166 | anti-mouse TCR β chain* | H57-597 | NA/LE | 0.5 mg |
| 553167 | anti-mouse TCR β chain* | H57-597 | Purified | 0.5 mg |
| 354720 | Mouse anti-CD3 T-Cell Activation Plates | 145-2C11 | NA/LE | 5 plates |

**Please see the BD Biosciences catalog or website for mAb to activate T cells bearing specific TCR Vα, β, γ, or δ chains.*

**Reagents for the study of CD28 family receptors and B7 family ligands in the mouse**

| | | | | |
|---|---|---|---|---|
| 553294 | anti-mouse CD28 | 37.51 | NA/LE | 0.5 mg |
| 557393 | anti-mouse CD28 | 37.51 | Purified | 0.1 mg |
| 553295 | anti-mouse CD28 | 37.51 | Purified | 0.5 mg |
| 553296 | anti-mouse CD28 | 37.51 | Biotin | 0.5 mg |
| 553297 | anti-mouse CD28 | 37.51 | PE | 0.2 mg |
| 553367 | anti-mouse CD80 (B7-1) | 1G10 | NA/LE | 0.5 mg |
| 553368 | anti-mouse CD80 (B7-1) | 1G10 | Purified | 0.5 mg |
| 553765 | anti-mouse CD80 (B7-1) | 16-10A1 | NA/LE | 0.5 mg |
| 553766 | anti-mouse CD80 (B7-1) | 16-10A1 | Purified | 0.5 mg |
| 553767 | anti-mouse CD80 (B7-1) | 16-10A1 | Biotin | 0.5 mg |
| 553768 | anti-mouse CD80 (B7-1) | 16-10A1 | FITC | 0.5 mg |
| 553769 | anti-mouse CD80 (B7-1) | 16-10A1 | PE | 0.2 mg |
| 553688 | anti-mouse CD86 (B7-2) | GL1 | NA/LE | 0.5 mg |
| 553689 | anti-mouse CD86 (B7-2) | GL1 | Purified | 0.5 mg |
| 553690 | anti-mouse CD86 (B7-2) | GL1 | Biotin | 0.5 mg |
| 553691 | anti-mouse CD86 (B7-2) | GL1 | FITC | 0.5 mg |
| 553692 | anti-mouse CD86 (B7-2) | GL1 | PE | 0.2 mg |
| 550542 | anti-mouse CD86 (B7-2) for immunohistochemistry | GL1 | Purified | 1 ml |
| 553838 | anti-mouse CD86 (B7-2) | PO3 | NA/LE | 0.5 mg |
| 558784 | anti-mouse CD86 (B7-2) | PO3 | Purified | 0.1 mg |
| 557426 | anti-mouse CD86 (B7-2) | PO3 | Biotin | 0.1 mg |
| 553718 | anti-mouse CD152 (CTLA-4) | UC10-4F10-11 | NA/LE | 0.5 mg |
| 553719 | anti-mouse CD152 (CTLA-4) | UC10-4F10-11 | Purified | 0.5 mg |
| 553720 | anti-mouse CD152 (CTLA-4) | UC10-4F10-11 | PE | 0.1 mg |
| 555278 | anti-mouse CD152 (CTLA-4) | 9H10 | NA/LE | 0.5 mg |
| 555279 | anti-mouse CD152 (CTLA-4) | 9H10 | Purified | 0.5 mg |
| 552015 | anti-mouse ICOS | 7E.17G9 | Purified | 0.5 mg |
| 551891 | anti-mouse PD-1 | J43 | Purified | 0.1 mg |
| 551892 | anti-mouse PD-1 | J43 | PE | 0.1 mg |
| 552132 | Mouse Non-cytolytic CTLA-4-IgG Fusion Protein | | NA/LE | 0.25 mg |
| 552133 | Mouse Non-cytolytic CTLA-4-IgG Fusion Protein | | NA/LE | 0.5 mg |

# NEW Reagents for Mouse T-Cell Costimulation *(continued from page 19)*

Table 2. BD Biosciences Reagents for T-Cell Costimulation Research *(continued)*

| Cat. No. | Description | Clone Name | Format | Size |
|---|---|---|---|---|
| **Reagents for TCR-mediated activation of rat T lymphocytes** | | | | |
| 554829 | anti-rat CD3 | G4.18 | NA/LE | 0.5 mg |
| 559974 | anti-rat CD3 | G4.18 | Purified | 0.1 mg |
| 554830 | anti-rat CD3 | G4.18 | Purified | 0.5 mg |
| 556970 | anti-rat CD3 | 1F4 | Purified | 0.5 mg |
| 554910 | anti-rat αβ TCR* | R73 | NA/LE | 0.5 mg |
| 554911 | anti-rat αβ TCR* | R73 | Purified | 0.5 mg |
| 554956 | anti-rat γδ TCR* | V65 | Purified | 0.5 mg |
| **Please see the BD Biosciences catalog or website for mAb to activate T cells bearing specific TCR Vα, β, γ, or δ chains.* | | | | |
| **Reagents for the study of CD28 family receptors and B7 family ligands in the rat** | | | | |
| 554992 | anti-rat CD28 | JJ316 | NA/LE | 0.5 mg |
| 554993 | anti-rat CD28 | JJ319 | NA/LE | 0.5 mg |
| 559982 | anti-rat CD28 | JJ319 | Purified | 0.1 mg |
| 550973 | anti-rat CD28 | JJ319 | FITC | 0.1 mg |
| 559984 | anti-rat CD28 | JJ319 | PE | 0.1 mg |
| 555012 | anti-rat CD80 (B7-1) | 3H5 | Purified | 0.5 mg |
| 555013 | anti-rat CD80 (B7-1) | 3H5 | Biotin | 0.5 mg |
| 555014 | anti-rat CD80 (B7-1) | 3H5 | PE | 0.2 mg |
| 555015 | anti-rat CD86 (B7-2)) | 24F | NA/LE | 0.5 mg |
| 555016 | anti-rat CD86 (B7-2)) | 24F | Purified | 0.5 mg |
| 555017 | anti-rat CD86 (B7-2)) | 24F | Biotin | 0.5 mg |
| 555018 | anti-rat CD86 (B7-2)) | 24F | FITC | 0.5 mg |
| 551396 | anti-rat CD86 (B7-2)) | 24F | PE | 0.1 mg |
| **Reagents for TCR-mediated activation of human T lymphocytes** | | | | |
| 555329 | anti-human CD3 | UCHT1 | NA/LE | 0.5 mg |
| 555330 | anti-human CD3 | UCHT1 | Purified | 0.1 mg |
| 555336 | anti-human CD3 | HIT3a | NA/LE | 0.5 mg |
| 555337 | anti-human CD3 | HIT3a | Purified | 0.1 mg |
| 354725 | Human anti-CD3 T-Cell Activation Plates | UCHT1 | NA/LE | 5 plates |
| 555546 | anti-human αβ TCR* | T10B9.1A-31 | Purified | 0.1 mg |
| **Please see the BD Biosciences catalog or website for mAb to activate T cells bearing specific TCR Vα, β, γ, or δ chains.* | | | | |
| **Reagents for the study of CD28 family receptors and B7 family ligands in the human** | | | | |
| 340975 | anti-human CD28 for immunohistochemistry | L293 | Purified | 0.2 mg |
| 348040 | anti-human CD28 | L293 | Purified | 0.5 mg |
| 348047 | anti-human CD28 | L293 | PE | 100 tests |
| 555725 | anti-human CD28 | CD28.2 | NA/LE | 0.5 mg |
| 555726 | anti-human CD28 | CD28.2 | Purified | 0.1 mg |
| 555727 | anti-human CD28 | CD28.2 | Biotin | 100 tests |
| 555728 | anti-human CD28 | CD28.2 | FITC | 100 tests |
| 555729 | anti-human CD28 | CD28.2 | PE | 100 tests |
| 555730 | anti-human CD28 | CD28.2 | Cy-Chrome™ | 100 tests |
| 559770 | anti-human CD28 | CD28.2 | APC | 100 tests |
| 550387 | anti-human CD28 for immunohistochemistry | CD28.2 | Purified | 1 ml |
| 340294 | anti-human CD80 | L307.4 | PE | 50 tests |
| 555681 | anti-human CD80 (BB1/B7-1) | BB1 | Purified | 0.1 mg |

Table 2. BD Biosciences Reagents for T-Cell Costimulation Research *(continued)*

| Cat. No. | Description | Clone Name | Format | Size |
|---|---|---|---|---|
| **Reagents for the study of CD28 family receptors and B7 family ligands in the human** *(continued)* | | | | |
| 555682 | anti-human CD80 (BB1/B7-1) | BB1 | Biotin | 100 tests |
| 555683 | anti-human CD80 (BB1/B7-1) | BB1 | FITC | 100 tests |
| 556058 | anti-human CD80 (BB1) | L307.4 | NA/LE | 0.5 mg |
| 557223 | anti-human CD80 (BB1) | L307.4 | Purified | 0.1 mg |
| 557225 | anti-human CD80 (BB1) | L307.4 | Biotin | 100 tests |
| 557226 | anti-human CD80 (BB1) | L307.4 | FITC | 100 tests |
| 557227 | anti-human CD80 (BB1) | L307.4 | PE | 100 tests |
| 559370 | anti-human CD80 (BB1) | L307.4 | Cy-Chrome | 100 tests |
| 555655 | anti-human CD86 (B70) | 2331(FUN-1) | Purified | 0.1 mg |
| 555656 | anti-human CD86 (B70) | 2331(FUN-1) | Biotin | 100 tests |
| 555657 | anti-human CD86 (B70) | 2331(FUN-1) | FITC | 100 tests |
| 555658 | anti-human CD86 (B70) | 2331(FUN-1) | PE | 100 tests |
| 555659 | anti-human CD86 (B70) | 2331(FUN-1) | Cy-Chrome | 100 tests |
| 555660 | anti-human CD86 (B70) | 2331(FUN-1) | APC | 100 tests |
| 555662 | anti-human CD86 (B70) | IT2.2 | NA/LE | 0.5 mg |
| 555663 | anti-human CD86 (B70) | IT2.2 | Purified | 0.1 mg |
| 555664 | anti-human CD86 (B70) | IT2.2 | Biotin | 100 tests |
| 555665 | anti-human CD86 (B70) | IT2.2 | PE | 100 tests |
| 555666 | anti-human CD86 (B70) | IT2.2 | Cy-Chrome | 100 tests |
| 555850 | anti-human CD152 (CTLA-4) | BNI3.1 | NA/LE | 0.5 mg |
| 555851 | anti-human CD152 (CTLA-4) | BNI3.1 | Purified | 0.1 mg |
| 555852 | anti-human CD152 (CTLA-4) | BNI3.1 | Biotin | 100 tests |
| 555853 | anti-human CD152 (CTLA-4) | BNI3.1 | PE | 100 tests |
| 555854 | anti-human CD152 (CTLA-4) | BNI3.1 | Cy-Chrome | 100 tests |
| 555855 | anti-human CD152 (CTLA-4) | BNI3.1 | APC | 100 tests |
| 550405 | anti-human CD152 for immunohistochemistry | BNI3.1 | Purified | 1 ml |
| **Reagents for TCR-mediated activation of non-human primate T lymphocytes** | | | | |
| 557052 | anti-human CD3ε | SP34 | NA/LE | 0.5 mg |
| 556610 | anti-human CD3ε | SP34 | Purified | 0.1 mg |
| **Reagents for the study of CD28 family receptors and B7 family ligands in the non-human primate** | | | | |
| 556620 | anti-human CD28 | CD28.2 | Purified | 0.1 mg |
| 556621 | anti-human CD28 | CD28.2 | FITC | 50 tests |
| 556622 | anti-human CD28 | CD28.2 | PE | 50 tests |
| 557146 | anti-human CD80 (BB1) | L307.4 | NA/LE | 0.1 mg |
| 557147 | anti-human CD80 (BB1) | L307.4 | PE | 50 tests |
| 557343 | anti-human CD86 (B70) | 2331(FUN-1) | FITC | 50 tests |
| 557344 | anti-human CD86 (B70) | 2331(FUN-1) | PE | 50 tests |
| 557300 | anti-human CD152 (CTLA-4) | BNI3.1 | Purified | 0.1 mg |
| 557301 | anti-human CD152 (CTLA-4) | BNI3.1 | PE | 50 tests |

*All reagents are for Research Use Only, not to be used in diagnostic or therapeutic procedures.*

# Immunohistochemistry in Neuroscience Research

By Padma Kodukula, Xiaokun Xiao, Xiang Dong Ji, and Joseph Voland

Modern studies in the functional organization of complex neuronal networks have facilitated our understanding of brain function. Many tools such as electron microscopy, immunofluorescence, confocal microscopy, immunohistochemistry, *in-situ* hybridization and use of tracers have contributed to our understanding of brain circuitry and how these interconnections correlate with brain function.

Immunohistochemistry is a powerful technique that allows the detection of neuronal connections in relation to the complete architecture of the brain. 25-30 µm free-floating brain sections are routinely used for this purpose, however, they often present researchers with several disadvantages including sub-optimal staining and poor morphology. In addition, the free floating brain sections staining technique requires large amounts of antibody and long incubation times. While microwave, formalin or paraformaldehyde fixation techniques have been used to facilitate immunohistochemical staining on paraffin embedded brain sections, many antibodies lose reactivity upon fixation with these techniques.

The Immunopathology department at BD Biosciences has developed an IHC Zinc fixative (Formalin-free) (Cat. No. 550523) for immunohistochemical staining, and we have now extended the use of this fixative to include our neuroscience antibodies. The morphology obtained is superior to that of free-floating sections and the Zinc fixative helps retain sample antigenicity. Over 80% of our portfolio of neuroscience antibodies from BD Biosciences Pharmingen and Transduction Laboratories work in our Zinc fixative, thus providing the researcher with a faster, easier, and more effective method of performing IHC staining in neural tissues. Detailed protocols and a complete line of secondary antibodies, isotype controls, and detection systems are available to perform immunohistochemical staining. In addition, we also have a large portfolio of antibodies to lymphoid markers, in human, mouse and rat, which are validated for use with our zinc fixative, allowing the simultaneous detection of both neuronal subtypes and inflammatory infiltrates in the same section.

In conclusion, BD Biosciences has a portfolio of more than 500 antibodies for the neuroscience researcher. Our new immunohistochemical staining technique utilizing Zinc-fixed 5 µm paraffin-embedded brain sections offers several advantages:

- Ease of Use
- Lower Amounts of Antibody Required
- Yields Superior Morphology
- Supported by a Complete Line of Ancillary Reagents

**References**

1. Evers P, Uylings HB, Suurmeijer AJ. Antigen retrieval in formaldehyde-fixed human brain tissue. Methods 1998 15:133-40
2. Van Dorp R, Boon ME, Kok PG, Marani E. Combining microwave stabilization and microwave-stimulated fixation of brain tissue with microwave-stimulated staining. Eur J Morphol 1995 33:164-73
3. Todd AJ. A method for combining confocal and electron microscopic examination of sections processed for double- or triple-labelling immunocytochemistry. J Neurosci Methods 1997; 73:149-57
4. Login GR, Dvorak AM. Application of microwave fixation techniques in pathology to neuroscience studies: a review. J Neurosci Methods 1994; 55:173-82
5. Hajos F, Garthwaite J, Garthwaite G, Csillag A Morphology of supravital brain slices pre-incubated in a physiological solution prior to fixation. Acta Morphol Hung 1989; 37:181-99

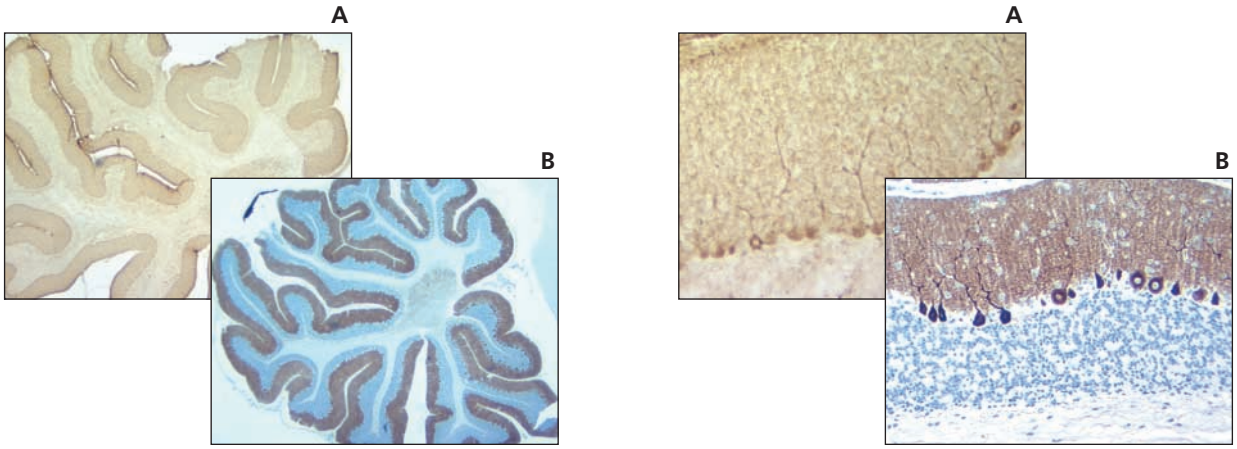
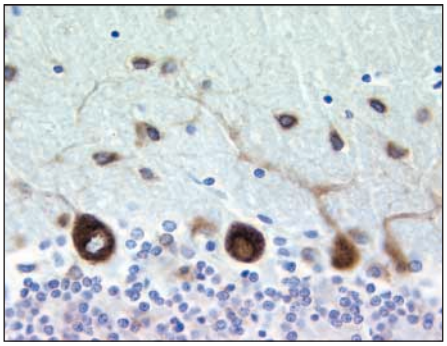
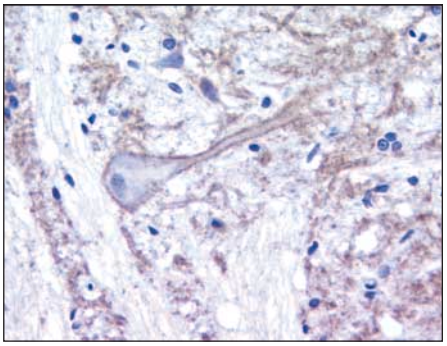


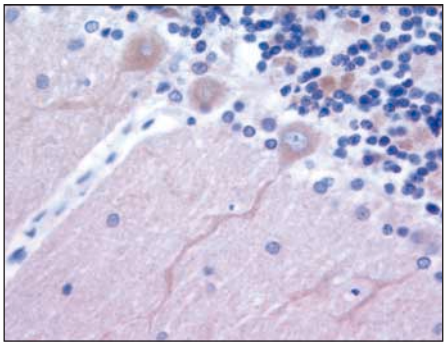

**Figure 1.** Glutamate Receptor 1α staining on normal rat cerebellum with free floating sections (A) and zinc fixed paraffin tissue (B). *Magnification 10X (left), 100X (right).*



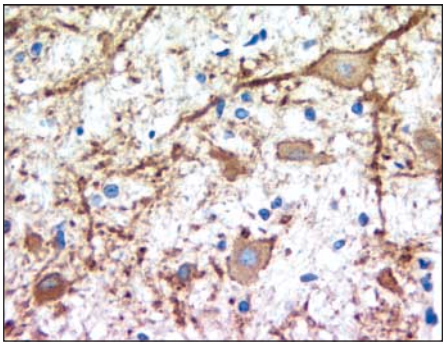
Acetylcholinesterase, rat cerebrum



Synaptogrin, rat cerebrum



Gephyrin, rat cerebellum



Clathrin heavy chain, rat cerebrum

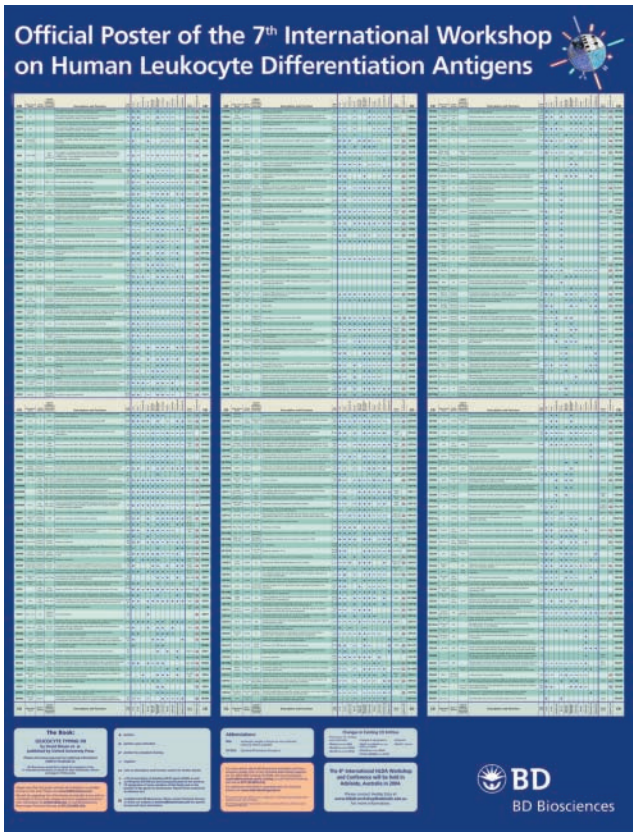
**Figure 2.** Additional neuronal markers on normal zinc fixed paraffin embedded brain.

# IHC Antibodies for Neuroscience Research Product List

| Cat. No. | Specificity | Clone | Isotype |
|---|---|---|---|
| 550523 | IHC Zinc Fixative (Formalin-free) | | |
| 610268 | Acetylcholinesterase | 46 | Mouse IgG1 |
| 610450 | ApoE | 32 | Mouse IgG1 |
| 610452 | B2 Bradykinin Receptor | 20 | Mouse IgG2b |
| 610260 | Calcineurin | 29 | Mouse IgG2a |
| 611293 | CaM Kinase II | 45 | Mouse IgG1 |
| 610276 | CaM Kinase IV | 26 | Mouse IgG1 |
| 611339 | Phospho-Caveolin (Y14) | 56 | Mouse IgG1 |
| 610500 | Clathrin Heavy Chain | 23 | Mouse IgG1 |
| 610204 | Cox-2 | 33 | Mouse IgG1 |
| 556313 | Dopamine b-hydroxylase | DBH 41 | Mouse IgG1 |
| 610246 | Dynamin | 41 | Mouse IgG1 |
| 610264 | Dynamin II | 27 | Mouse IgG2a |
| 610675 | Dyrk | 106 | Mouse IgG1 |
| 610781 | Dystrobrevin | 38 | Mouse IgG1 |
| 610124 | ERK (pan ERK) | 16 | Mouse IgG2a |
| 610031 | ERK1 | MK12 | Mouse IgG1 |
| 610104 | ERK2 | 33 | Mouse IgG2b |
| 610585 | Gephyrin | 45 | Mouse IgG1 |
| 556389 | Glutamate Receptor (mGluR1a) | G209-2048 | Mouse IgG1 |
| 556331 | Glutamate Receptor (mGluR1a) | G209-488 | Mouse IgG1 |
| 610518 | Glutamine Synthetase | 6 | Mouse IgG2a |
| 610202 | GSK-3b | 7 | Mouse IgG1 |
| 610327 | Jun | 3 | Mouse IgG2a |
| 610693 | Mena | 21 | Mouse IgA |
| 611029 | Mint1 | 23 | Mouse IgG1 |
| 611033 | Mint2 | 18 | Mouse IgG1 |
| 610337 | Munc18 | 31 | Mouse IgG1 |
| 556309 | Nestin | Rat 401 | Mouse IgG1 |
| 610372 | Neuronal Pentraxin | Polyclonal | Rabbit Ig |
| 610777 | Ninjurin | 50 | Mouse IgG2a |
| 610309 | nNOS/NOS Type 1 | 16 | Mouse IgG2a |
| 610474 | p150Glued | 1 | Mouse IgG1 |
| 610863 | Pax-5 | 24 | Mouse IgG1 |
| 610398 | PKC d | 14 | Mouse IgG2b |
| 610086 | PKC e | 21 | Mouse IgG2a |
| 610556 | PP2A Catalytic a | 46 | Mouse IgG1 |
| 610549 | PYK2 (CAK b) | 11 | Mouse IgG1 |
| 610178 | RACK1 | 20 | Mouse IgM |
| 556326 | Serotonin Receptor 5-HT2AR | G186-1117 | Mouse IgG1 |
| 610647 | SMN | 8 | Mouse IgG1 |
| 610667 | Synapsin IIa | 1 | Mouse IgG1 |
| 610598 | Synaptogyrin | 6 | Mouse IgG1 |
| 610636 | Syntaxin 6 | 30 | Mouse IgG1 |
| 556311 | Tyrosine Hydroxylase | TOH A1 | Mouse IgG1 |

## HLDA Poster Now Available from BD Biosciences

The Official Poster of the 7th International Workshop on Human Leukocyte Differentiation Antigens (HLDA) is *now available* from BD Biosciences.




**The 8th HLDA meeting will be held in Australia in 2004.**

For more information email **hlda8.workshop@adelaide.edu.au** or visit **http://www.hlda8.org/**

# NEW BD RiboQuant™ Non-Rad Ribonuclease Protection Assay (RPA)

By Susan Chambers

Our NEW version of the BD RiboQuant™ Ribonuclease Protection Assay (RPA) offers a non-radiolabeled approach to this powerful and versatile system. The Multi-Probe RPA system provides a highly sensitive and specific method of multiplex gene expression analysis, and has traditionally been performed using probes labeled by the incorporation of [32P]-UTP to provide optimal signal and sensitivity. By using our new kit to generate non-radiolabeled probes for multi-probe RPA, risks associated with exposure to radioactivity, environmental concerns and regulatory requirements are eliminated. With our non-radioactive kits, researchers can now choose which system best suits their needs.

The BD RiboQuant Multi-Probe RPA system is a well-established method to detect and quantitate up to 13 mRNA species simultaneously from a single sample of total RNA. The Multi-Probe RPA is made possible by generating a series of templates, each of a different length and each representing a sequence in a distinct mRNA species. The templates are assembled into biologically relevant sets and used for the T7 polymerase-directed synthesis of a high-specific-activity, biotin labeled, anti-sense RNA probe set. The probe set is then hybridized, in excess, to target RNA in solution. Following hybridization, free probe and other single-stranded RNA are digested with RNases. The remaining "RNase-protected" probes are purified, resolved on denaturing polyacrylamide gels, then transferred to a positively-charge nylon membrane. The membrane is probed and the signal quantified using Streptavidin-HRP and the enhanced chemiluminescent substrate provided in the kit. The membrane is exposed to x-ray film or a CCD camera and the level of each mRNA species in the original RNA sample is determined based on the intensity of the appropriately-sized, protected probe fragment (Figure 1).

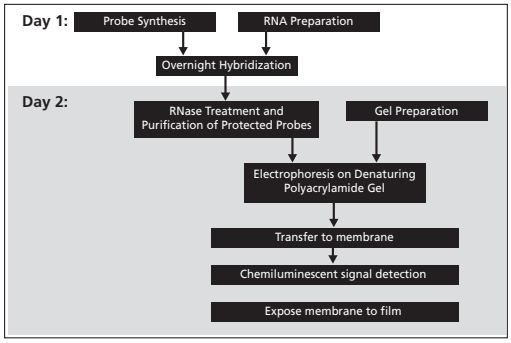



Figure 1

### Unparalleled Range of Specificities Available

We offer over 100 pre-assembled, biologically relevant multi-probe template sets and over 1000 specific templates from which to choose. We also offer custom template construction and/or assembly to meet your needs.

## The BD RiboQuant™ Non-Rad RPA System includes:

| Multi-Probe Template Sets | Cat. No. |
|---|---|
| BD RiboQuant Non-Rad *In Vitro* Transcription Kit* | 551917 |
| BD RiboQuant RPA Kit | 556134 |
| BD RiboQuant Non-Rad Detection Kit | 551918 |
| BD RiboQuant Non-Rad Starter Package† | 551919 |

*The BD RiboQuant Non-Rad In Vitro Transcription Kit is optimized for the efficient synthesis of high-specific-activity, biotin-labeled riboprobes from the BD Pharmingen™ Multi-Probe Template Sets. Each kit contains sufficient reagents for 5 transcription reactions, yielding ~10 µg biotin-labeled probe per reaction.*

*The BD RiboQuant Non-Rad Detection Kit includes 10 positively-charged nylon membranes and all reagents necessary for signal development using enhanced chemiluminescence.*

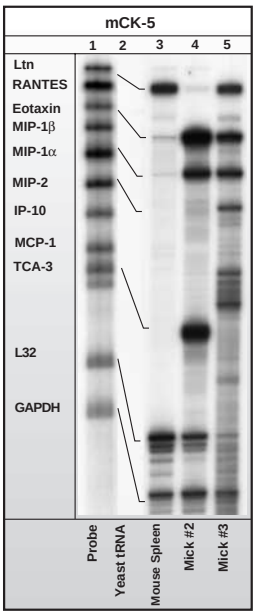
† *Individual kits or sets of the system may be purchased separately or together, with one Multi-Probe Template Set of choice, as the BD RiboQuant Non-Rad RPA Starter Package, Cat. No. 551919.*

* *Biotinylated nucleotides must be purchased separately. BD RiboQuant non-radioactive RPA has been optimized with biotin-16-UTP available from Roche Biosciences.*

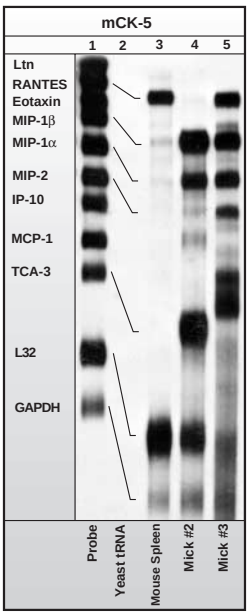
*All protocols are described in the newest edition of the BD RiboQuant Instruction Manual.*

## RNase Protection Assays Comparing Radiolabeled Probes and Biotin-labeled Probes

**Radiolabeled Probes**




**Biotin-labeled Probes**




**mCK-5**

Cat. No. 556146

| Template | Probe | Protected |
|---|---|---|
| Ltn | 390 | 361 |
| RANTES | 349 | 321 |
| Eotaxin | 314 | 285 |
| MIP-1β | 285 | 257 |
| MIP-1α | 256 | 228 |
| MIP-2 | 231 | 205 |
| IP-10 | 210 | 181 |
| MCP-1 | 190 | 163 |
| TCA-3 | 171 | 143 |
| L32 | 141 | 112 |
| GAPDH | 125 | 97 |

RPA was performed using 10 µg total RNA from various cell populations: (Lane 3) untreated mouse spleen. (Lane 4) Purified $CD4^+$ splenocytes stimulated with anti-mouse CD3 and anti-mouse CD28, plus purified mouse IL-2 and mouse IL-4, followed by restimulation with PMA + ionomycin. (Lane 5) 3-day thioglycolate-elicited peritoneal cells stimulated with purified mouse IFN-γ and LPS in the presence of a protein transport inhibitor. *(For complete stimulation protocols, please refer to Cytokines/Chemokines Application Manual, 3rd ed., Pharmingen, June 1999, pp31-32.)*

### BD RiboQuant™ Non-Rad *In Vitro* Transcription Kit (551917)

| Reagent | Concentration | Volume | Reactions |
|---|---|---|---|
| 5x Nucleotide Mix | 5X ( 5 mM A,C,G 3.25 mM UTP) | 16.5 µl | 5 |
| 5x Transcription Buffer | 5X | 20 µl | 5 |
| DTT | 100 mM | 10 µl | 5 |
| Enzyme Mix (RNasin & T7 RNA Polymerase) | RNasin (40 U/ml) T7 RNA Polymerase (20 U/ml) | 10 µl | 5 |
| RNase-free DNase I | 1 U/ml | 10 µl | 5 |
| EDTA | 20 mM | 135 µl | 5 |
| Glycogen | 5 mg / ml | 5 µl | 5 |
| LiCl | 4 M | 2.7 ml | 5 IVT, 200 RPA |

### BD RiboQuant™ Non-Rad Detection Kit (551918)

| Reagent | Volume | Reactions |
|---|---|---|
| Nylon Membrane | | 10 |
| Hybridization Buffer | 100 ml | 10 |
| Hybridization Stringency Wash Buffer | 300 ml | 10 |
| Membrane Blocking Buffer | 400 ml | 10 |
| Wash Buffer (4X) | 400 ml | 10 |
| Substrate Equilibration Buffer | 400 ml | 10 |
| Streptavidin-Horseradish Peroxidase | 1.5 ml | 10 |
| Stable Peroxide Buffer | 50 ml | 10 |
| Luminol / Enhancer | 50 ml | 10 |

# NEW Products Available Since the 2001/2002 BD Biosciences Catalog

| Cat No. | Description | Clone | Format | Size |
|---|---|---|---|---|
| **Cell Biology Reagents** | | | | |
| 551456 | Acinus | Rabbit pAb | Purified | 100 ul |
| 551150 | Act Casp-3 Poly (PAb CM1) | | Serum | 100 µl |
| 551429 | AIF (Apoptosis-Inducing Factor) | Rabbit pAb | Purified | 100 ul |
| 612082 | α-Methylacyl-CoA Racemase | 15 | Purified | 50 ug |
| 612083 | α-Methylacyl-CoA Racemase | 15 | Purified | 150 ug |
| 550651 | Aquaporin 1 | Rabbit pAb | Purified | 50 ul |
| 550650 | Aquaporin 1 | Rabbit pAb | Purified | 200 ul |
| 550649 | Aquaporin 2 | Rabbit pAb | Purified | 50 ul |
| 550648 | Aquaporin 2 | Rabbit pAb | Purified | 200 ul |
| 550647 | Aquaporin 3 | Rabbit pAb | Purified | 50 ul |
| 550646 | Aquaporin 3 | Rabbit pAb | Purified | 200 ul |
| 612072 | ASAP1 | 19 | Purified | 50 ug |
| 612073 | ASAP1 | 19 | Purified | 150 ug |
| 551432 | BACE (β-site APP Cleaving Enzyme) | Rabbit pAb | Purified | 100 ul |
| 612110 | BAF47 | 25 | Purified | 50 ug |
| 612111 | BAF47 | 25 | Purified | 150 ug |
| 612112 | Beclin | 20 | Purified | 50 ug |
| 612113 | Beclin | 20 | Purified | 150 ug |
| 551283 | Blys/BAFF | | Purifed | 200 µl |
| 550719 | Ca2+channel α1A | | Purified | 200 µl |
| 550717 | Ca2+channel α1B | | Purified | 200 µl |
| 550715 | Ca2+channel α1C | | Purified | 200 µl |
| 550711 | Ca2+channel α1D | | Purified | 200 µl |
| 550709 | Ca2+channel α1E | | Purified | 200 µl |
| 550705 | Ca2+channel Ca β3 | | Purified | 200 µl |
| 550703 | Ca2+channel Caγ3 | | Purified | 200 µl |
| 550706 | Ca2+channel Ca-β-3 | | Purified | 50 µl |
| 550704 | Ca2+channel Ca-γ-2 | | Purified | 50 µl |
| 550716 | Ca2+channel CaV1.2 | | Purified | 50 µl |
| 550712 | Ca2+channel CaV1.3 | | Purified | 50 µl |
| 550720 | Ca2+channel CaV2.1 | | Purified | 50 µl |
| 612130 | E-Cadherin | 36 | FITC | 50 ug |
| 612131 | E-Cadherin | 36 | FITC | 150 ug |
| 611786 | Human Carcinoma I Lysate Kit | | Lysate | 1 Kit |
| 551241 | Caspase-7 | 11-1-56.1 | Purified | 150 ug |
| 551240 | Caspase-7 | 11-1-56.1 | Purified | 50 ug |
| 551239 | Caspase-7 | 10-1-62.1 | Purified | 150 µg |
| 551237 | Caspase-7 | 8-1-47.1 | Purified | 150 µg |
| 551243 | Caspase-8 | 3-1-9.1.1 | Purified | 150 µg |
| 551245 | Caspase-8 | 4-1-20.1 | Purified | 150 µg |
| 551247 | Caspase-9 | 2-22.1 | Purified | 150 µg |
| 551430 | Caspase-12 | Rabbit pAb | Purified | 100 ul |
| 551443 | Caspase-13 | Rabbit pAb | Purified | 100 ul |
| 550873 | Caspase-14 | 70A1426 | Purified | 150 ug |
| 551526 | Cdk1/Cdk2 | AN21.2 | Purified | 50 ug |
| 551527 | Cdk1/Cdk2 | AN21.2 | Purified | 150 ug |
| 612074 | Coilin | 56 | Purified | 50 ug |
| 612075 | Coilin | 56 | Purified | 150 ug |
| 612076 | Collybistin | 3 | Purified | 50 ug |
| 612077 | Collybistin | 3 | Purified | 150 ug |
| 612078 | CRP2 | 1 | Purified | 50 ug |
| 612079 | CRP2 | 1 | Purified | 150 ug |
| 551547 | D4-GDI-FITC | 97A1015 | FITC | 50 ug |
| 551546 | D4-GDI-PE | 97A1015 | PE | 50 ug |
| 611911 | Daudi Cell Lysate | | Lysate | 500 ug |
| 550945 | DRAK2 | | Purified | 200 ul |
| 612090 | β-Dystroglycan | 56 | Purified | 50 ug |
| 612091 | β-Dystroglycan | 56 | Purified | 150 ug |
| 612114 | EPLIN | 20 | Purified | 50 ug |

## Cell Biology Reagents *(continued)*

| Cat No. | Description | Clone | Format | Size |
|---|---|---|---|---|
| 612115 | EPLIN | 20 | Purified | 150 ug |
| 551527 | Fractin (Cleaved Actin) | Rabbit pAb | Purified | 100 ul |
| 612092 | GRASP55 | 21 | Purified | 50 ug |
| 612093 | GRASP55 | 21 | Purified | 150 ug |
| 611872 | HDJ-2 | 30 | Purified | 50 ug |
| 611873 | HDJ-2 | 30 | Purified | 150 ug |
| 611886 | Headpin | 31 | Purified | 50 ug |
| 611887 | Headpin | 31 | Purified | 150 ug |
| 611888 | Headpin | 49 | Purified | 50 ug |
| 611889 | Headpin | 49 | Purified | 150 ug |
| 612066 | HepG2+ IL-6 Ctrl Lysate | | Lysate | 500 ug |
| 612067 | HepG2+IL-6 (15') Lysate | | Lysate | 500 ug |
| 612118 | Hip1R | 44 | Purified | 50 ug |
| 612119 | Hip1R | 44 | Purified | 150 ug |
| 551818 | IKBα, Phospho-Specific | 39A1413 | Purified | 50 ug |
| 612120 | JAM-1 | 43 | Purified | 50 ug |
| 612121 | JAM-1 | 43 | Purified | 150 ug |
| 550959 | Jurkat Apoptotic Lysate Set I | Set | Lysate | 1 mg |
| 550687 | K+ Channel Kv1.2 | Rabbit pAb | Purified | 200 ul |
| 550685 | K+ Channel Kv1.3 | Rabbit pAb | Purified | 200 ul |
| 550680 | K+ Channel Kv1.6 | Rabbit pAb | Purified | 50 ul |
| 550679 | K+ Channel Kv1.6 | Rabbit pAb | Purified | 200 ul |
| 550678 | K+ Channel Kv2.1 | Rabbit pAb | Purified | 50 ul |
| 550677 | K+ Channel Kv2.1 | Rabbit pAb | Purified | 200 ul |
| 550675 | K+ Channel Kv3.1b | Rabbit pAb | Purified | 50 ul |
| 550673 | K+ Channel Kv3.1b | Rabbit pAb | Purified | 200 ul |
| 550655 | K+ Channel SK3 | Rabbit pAb | Purified | 50 ul |
| 550654 | K+ Channel SK3 | Rabbit pAb | Purified | 200 ul |
| 550690 | K+ Channel Kv1.1 | Rabbit pAb | Purified | 50 µl |
| 550689 | K+ Channel Kv1.1 | Rabbit pAb | Purified | 200 µl |
| 550671 | K+ Channel Kv3.2 | Rabbit pAb | Purified | 50 µl |
| 550670 | K+ Channel Kv3.2 | Rabbit pAb | Purified | 200 µl |
| 550667 | K+ Channel Kv4.2 | Rabbit pAb | Purified | 50 µl |
| 550666 | K+ Channel Kv4.2 | Rabbit pAb | Purified | 200 µl |
| 612094 | KIF1A | 16 | Purified | 50 ug |
| 612096 | KIF2 | 7 | Purified | 50 ug |
| 612097 | KIF2 | 7 | Purified | 150 ug |
| 612080 | MAPKAPK-5 | 50 | Purified | 50 ug |
| 612081 | MAPKAPK-5 | 50 | Purified | 150 ug |
| 612100 | Neurotensin Receptor 3 | 48 | Purified | 50 ug |
| 612101 | Neurotensin Receptor 3 | 48 | Purified | 150 ug |
| 612098 | NSP1 | 6 | Purified | 50 ug |
| 612099 | NSP1 | 6 | Purified | 150 ug |
| 550871 | Nucleosome | 6E5 | Purified | 150 ug |
| 550779 | Nucleosome | 11E6 | Purified | 150 µg |
| 550699 | Purinergic Receptor P2X1 | Rabbit pAb | Purified | 200µl |
| 550700 | Purinergic Receptor P2X1 | Rabbit pAb | Purified | 50 µl |
| 550698 | Purinergic Receptor P2X4 | Rabbit pAb | Purified | 50 µl |
| 550697 | Purinergic Receptor P2X4 | Rabbit pAb | Purified | 200 µl |
| 550692 | Purinergic Receptor P2Y4 | Rabbit pAb | Purified | 50 µl |
| 550691 | Purinergic Receptor P2Y4 | Rabbit pAb | Purified | 200 µl |
| 551358 | Purinergic Receptor PAK4 | E440-883 | Purified | 50 ug |
| 550694 | Purinergic Receptor P2X7 | Rabbit pAb | Purified | 50 ul |
| 550693 | Purinergic Receptor P2X7 | Rabbit pAb | Purified | 200 ul |
| 551528 | PARP (Cleavage Site-Specific) | Rabbit pAb | Purified | 200 ul |
| 612084 | Peroxiredoxin V | 44 | Purified | 50 ug |
| 612085 | Peroxiredoxin V | 44 | Purified | 150 ug |
| 612102 | Phosphatase Methylesterase-1 | 8 | Purified | 50 ug |
| 612103 | Phosphatase Methylesterase-1 | 8 | Purified | 150 ug |

# NEW Products from BD Biosciences *(continued from page 29)*

| Cat No. | Description | Clone | Format | Size |
|---|---|---|---|---|
| **Cell Biology Reagents** *(continued)* | | | | |
| 551431 | RAIDD/CRADD | Rabbit pAb | Purified | 100 ul |
| 612086 | SCAMP1 | 22 | Purified | 50 ug |
| 612087 | SCAMP1 | 22 | Purified | 150 ug |
| 612088 | Selenocysteine Lyase | 32 | Purified | 50 ug |
| 612089 | Selenocysteine Lyase | 32 | Purified | 150 ug |
| 612104 | SGT1 | 29 | Purified | 50 ug |
| 612105 | SGT1 | 29 | Purified | 150 ug |
| 551821 | Smac/DIABLO | Rabbit pAb | Serum | 100 ul |
| 511816 | Syk | 4D10 | Purified | 50 ug |
| 511817 | Syk | 4D10 | Purified | 150 ug |
| 612122 | TIP120 | 48 | Purified | 50 ug |
| 612123 | TIP120 | 48 | Purified | 150 ug |
| 551357 | TOAD-64 | Rabbit pAb | Serum | 100 ul |
| 551316 | TROP-1 | 162-21 | Purified | 0.1 mg |
| 551317 | TROP-2 | 162-46 | Purified | 0.1 mg |
| 550701 | TRPC1 | | Purified | 200 µl |
| 550702 | TRPC1 | | Purified | 50 µl |
| 612106 | Tubby | 40 | Purified | 50 ug |
| 612107 | Tubby | 40 | Purified | 150 ug |
| 550944 | Ubiquitin | 6C1.17 | Ascites | 100 ul |
| 612126 | XPF | 26 | Purified | 50 ug |
| 612127 | XPF | 26 | Purified | 150 ug |
| 612108 | ZIP Kinase | 17 | Purified | 50 ug |
| 612109 | ZIP Kinase | 17 | Purified | 150 ug |
| 612128 | ZPR1 | 8 | Purified | 50 ug |
| 612129 | ZPR1 | 8 | Purified | 150 ug |
| **Cytokine and Chemokine Reagents** | | | | |
| **Antibodies for Sandwich ELISA** | | | | |
| 551403 | Rat anti-human sCD14 (ELISA Capture) | 55-3 | Purified | 0.5 mg |
| 551405 | Mouse anti-human sCD14 (ELISA Detection) | 3-C39 | Biotin | 0.5 mg |
| 551388 | Mouse anti-human sIL-1RI (ELISA Capture) | hIL-1R-M1 | Purified | 0.5 mg |
| 551389 | Mouse anti-human sIL-1RI (ELISA Detection) | hIL-1R-M8 | Biotin | 0.5 mg |
| 550823 | Mouse anti-pig IL-1β (ELISA Capture) | 4B2. | Purified | 0.5 mg |
| 550824 | Mouse anti-pig IL-1β (ELISA Detection) | 6E8.10 | Biotin | 0.5 mg |
| 550604 | Hamster anti-mouse IL-1α (ELISA Capture) | ALF-161 | Purified | 0.5 mg |
| 550606 | Rabbit anti-mouse IL-1α (ELISA Detection) | Rabbit Ig | Biotin | 0.5 mg |
| 550605 | Hamster anti-mouse IL-1β (ELISA Capture) | B122 | Purified | 0.5 mg |
| 550623 | Rabbit anti-mouse IL-1β (ELISA Detection) | Rabbit Ig | Biotin | 0.5 mg |
| 551309 | Rat anti-mouse IFNγ (ELISA Capture) | AN-18 | Purified | 0.5 mg |
| 551506 | Rat anti-mouse IFNγ (ELISA Detection) | R4-6A2 | Biotin | 0.5 mg |
| 552035 | FUT-175 (Futhan) Protease Inhibitor for Complement Measurements | _ | | 5 mg |
| **Antibodies for Staining and Flow Cytometric Analysis** | | | | |
| 551087 | Mouse anti-human C1qRp | R139 | Purified | 0.1 mg |
| 551531 | Mouse anti-human C1qRp | R139 | PE | 0.1 mg |
| 551509 | Mouse anti-human C1qRp | R139 | FITC | 0.2 mg |
| 551454 | Mouse anti-human C1qRp | R3 | Purified | 0.1 mg |
| 552117 | Mouse anti-human C1qRp | R3 | Biotin | 100 tests |
| 552032 | Rat anti-human CXCR5 | RF8B2 | Purified | 0.1 mg |
| 552118 | Rat anti-human CXCR5 | RF8B2 | Biotin | 100 tests |
| 551284 | Mouse anti-human GM-CSFRa | hGMCSFR-M1 | Purified | 0.5 mg |
| 551412 | Mouse anti-human GM-CSFRa | hGMCSFR-M1 | Biotin | 0.5 mg |
| 551373 | Mouse anti-human GM-CSFRa | hGMCSFR-M1 | PE | 0.2 mg |
| 551462 | Mouse anti-human IL-6Rα | M5 | Purified | 0.5 mg |
| 551851 | Mouse anti-human IL-6Rα | M5 | Biotin | 100 tests |
| 551850 | Mouse anti-human IL-6Rα | M5 | PE | 100 tests |

## Cytokine and Chemokine Reagents *(continued)*

### Antibodies for Staining and Flow Cytometric Analysis *(continued)*

| Cat No. | Description | Clone | Format | Size |
|---|---|---|---|---|
| 551894 | Mouse anti-human IL-4Rα | hIL4R-M57 | Purified | 0.1 mg |
| 552120 | Mouse anti-human IL-4Rα | hIL4R-M57 | Biotin | 100 tests |
| 550900 | Mouse anti-human TNFRI | MABTNFR1-B1 | Biotin | 0.5 mg |
| 551359 | Mouse anti-human TNFR Related Protein (LTbR) | hTNFR-RP-M12 | Purified | 0.5 mg |
| 551861 | Mouse anti-human TNFR Related Protein (LTbR) | hTNFR-RP-M12 | Biotin | 100 tests |
| 551503 | Mouse anti-human TNFR Related Protein (LTbR) | hTNFR-RP-M12 | PE | 0.2 mg |
| 551311 | Rat anti-human TNFRII | hTNFR-M1 | Purified | 0.5 mg |
| 551964 | Mouse anti-human Toll-like receptor 4 | HTA125 | Purified | 0.1 mg |
| 551975 | Mouse anti-human Toll-like receptor 4 | HTA125 | Biotin | 100 tests |
| 552034 | Mouse anti-human Toll-like receptor 4 | HTA125 | PE | 100 tests |
| 552033 | Mouse anti-human Toll-like receptor 1 | GD2 | Purified | 0.1 mg |
| 551961 | Rat anti-mouse CXCR5 | 2G8 | Purified | 0.1 mg |
| 551960 | Rat anti-mouse CXCR5 | 2G8 | Biotin | 0.1 mg |
| 551959 | Rat anti-mouse CXCR5 | 2G8 | PE | 0.1 mg |
| 551852 | Rat anti-mouse CXCR4 | 2B11/CXCR4 | Purified | 0.1 mg |
| 551968 | Rat anti-mouse CXCR4 | 2B11/CXCR4 | Biotin | 0.1 mg |
| 551966 | Rat anti-mouse CXCR4 | 2B11/CXCR4 | PE | 0.1 mg |
| 551967 | Rat anti-mouse CXCR4 | 2B11/CXCR4 | FITC | 0.1 mg |
| 551970 | Rat anti-human CXCR4 | 1D9 | Biotin | 100 tests |
| 551853 | Rat anti-mouse IL-4R | IL4R-M1 | Purified | 0.1 mg |
| 551455 | Mouse anti-mouse IL-12Rβ1 | 114 | Purified | 0.5 mg |
| 551973 | Mouse anti-mouse IL-12 Rβ1 | 114 | Biotin | 0.1 mg |
| 551974 | Mouse anti-mouse IL-12 Rβ1 | 114 | PE | 0.1 mg |
| 551074 | Mouse IgG2a isotype control | G155-178 | Biotin | 0.25 mg |

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

| | | | | |
|---|---|---|---|---|
| 552052 | Mouse anti-human CD14 | 3-C39 | NA/LE | 0.5 mg |
| 552119 | Mouse anti-human GM-CSFRα | hGMCSFR-M1 | NA/LE | 0.5 mg |
| 551795 | Mouse anti-human IFN-α | 7N4-1 | NA/LE | 0.5 mg |
| 551963 | Mouse anti-human Toll-like receptor 4 | HTA125 | NA/LE | 0.5 mg |
| 557538 | Hamster anti-mouse IL-1R1 | Jama-141 | NA/LE | 0.25 mg |
| 550877 | Mouse anti-rabbit IL-8 | 2g3 | NA/LE | 0.5 mg |

## BD OptEIA™ CL ELISA Kits (Chemiluminescent)

| | | | |
|---|---|---|---|
| 551794 | Human IL-2 Chemiluminescent Kit | Kit | 1 plate |
| 551501 | Human IFN-γ Chemiluminescent Kit | Kit | 1 plate |
| 551502 | Human TNF-α Chemiluminescent Kit | Kit | 1 plate |

## BD OptEIA™ ELISA Kits (Colorimetric)

| | | | |
|---|---|---|---|
| 550947 | Human C4a-desArg | Kit | 1 plate |
| 550611 | Human IL-2 Kit II | Kit | 2 plates |
| 550614 | Human IL-4 Kit II | Kit | 2 plates |
| 550949 | Human IL-5 Kit II | Kit | 2 plates |
| 550799 | Human IL-6 Kit II | Kit | 2 plates |
| 550999 | Human IL-8 Kit II | Kit | 2 plates |
| 550613 | Human IL-10 Kit | Kit | 2 plates |
| 550801 | Mouse IL-2 Kit | Kit | 2 plates |
| 550997 | Mouse IL-5 Kit | Kit | 2 plates |
| 550950 | Mouse IL-6 Kit | Kit | 2 plates |
| 551423 | Mouse IL-12 p40 Kit | Kit | 2 plates |

## BD OptEIA™ Sets (Colorimetric)

| | | | |
|---|---|---|---|
| 550926 | Human IP-10 Set | Set | Rgts for 20 plates |
| 551424 | Human sICAM-1 Set | Set | Rgts for 20 plates |
| 550995 | Human TNF-β Set | Set | Rgts for 20 plates |
| 550996 | Human TNFRI Set | Set | Rgts for 20 plates |
| 550948 | Human TRAIL Set | Set | Rgts for 20 plates |

# NEW Products from BD Biosciences *(continued from page 31)*

| Cat No. | Description | Clone | Format | Size |
|---|---|---|---|---|
| **BD OptEIA™ Sets (Colorimetric)** *(continued)* | | | | |
| 551492 | Monkey IFN-γ Set | | Set | Rgts for 20 plates |
| 551494 | Monkey IL-2 Set | | Set | Rgts for 20 plates |
| 551495 | Monkey IL-4 Set | | Set | Rgts for 20 plates |
| 551496 | Monkey IL-6 Set | | Set | Rgts for 20 plates |
| 551493 | Monkey TNF-α Set | | Set | Rgts for 20 plates |
| 551866 | Mouse IFN-γ Set (AN-18) | | Set | Rgts for 20 plates |
| 559753 | Mouse MIP-1b Set | | Set | Rgts for 20 plates |
| 550319 | Rat IL-6 Set | | Set | Rgts for 20 plates |
| **Non-Human Primate Reagents** | | | | |
| 551466 | CD4/CCR5 | M-T477/3A9 | Multicolor | 50 test |
| 551465 | CD4/CXCR4 | M-T477/12G5 | Multicolor | 50 test |
| 551470 | CD8/CCR5 | RPA-T8/3A9 | Multicolor | 50 test |
| 551468 | CD8/CXCR4 | RPA-T8/12G5 | Multicolor | 50 test |
| 551467 | mIgG1/mIgG2a | MOPC-21/G155-178 | Multicolor | 50 test |
| 551464 | mIgG2a/mIgG2a | G155-178/G155-178 | Multicolor | 50 test |
| 557341 | Mouse anti-human CD62L | SK11 (leu8) | PE | 50 test |
| 551300 | Mouse anti-baboon RBC | E34-731 | FITC | 50 test |
| **Human Cell Surface Reagents** | | | | |
| 551082 | Mouse anti-human β7 integrin | FIB504 | APC | 100 tests |
| 551059 | Mouse anti-human β7 integrin | FIB504 | Cy-Chrome | 100 tests |
| 551337 | Mouse anti-human β2-microglobulin | TÜ99 | PE | 100 tests |
| 551340 | Mouse anti-human Bcl-10 | 151 | Purified | 0.1 mg |
| 550796 | Mouse anti-human CD3 | UCHT1 | PerCP-Cy5.5 | 0.1 mg |
| 550788 | Mouse anti-human CD4 | RPA-T4 | PerCP-Cy5.5 | 0.1 mg |
| 550791 | Mouse anti-human CD8 | RPA-T8 | PerCP-Cy5.5 | 0.1 mg |
| 551131 | Mouse anti-human CD11a/LFA-1 | HI111 | Cy-Chrome | 100 tests |
| 550787 | Mouse anti-human CD14 | M5E2 | PerCP-Cy5.5 | 0.1 mg |
| 551060 | Mouse anti-human CD18 | 6.7 | APC | 100 tests |
| 550789 | Mouse anti-human CD19 | HIB19 | PerCP-Cy5.5 | 0.1 mg |
| 551064 | Mouse anti-human CD21 | B-ly4 | Cy-Chrome | 100 tests |
| 551444 | Mouse anti-human CD34 | 581 | PerCP-Cy5.5 | 0.1 mg |
| 551141 | Mouse anti-human CD42b | HIP1 | Cy-Chrome | 100 tests |
| 550988 | Mouse anti-human CD44 | 515 | Purified | 0.1 mg |
| 551140 | Mouse anti-human CD49f | GoH3 | APC | 100 tests |
| 550813 | Mouse anti-human Cw60 | UM-4D4 | FITC | 100 tests |
| 551374 | Mouse anti-human CD71 | M-A712 | APC | 100 tests |
| 551134 | Mouse anti-human CD79a | HM47 | APC | 100 tests |
| 551063 | Mouse anti-human CD79b | CB3-1 | Cy-Chrome | 100 tests |
| 550955 | Mouse anti-human CD79b | CB3-1 | APC | 100 tests |
| 551112 | Mouse anti-human CD81 | JS-81 | APC | 100 tests |
| 551053 | Mouse anti-human CD85 | GH1/75 | PE | 100 tests |
| 551054 | Mouse anti-human CD85 | GHI/75 | Cy-Chrome™ | 100 tests |
| 551081 | Mouse anti-human CDw128 | 5A12 | Cy-Chrome™ | 100 tests |
| 551137 | Mouse anti-human CDw137 | 4B4-1 | Cy-Chrome | 100 tests |
| 551902 | Mouse anti-human CD138 | Mi15 | Purified | 0.1 mg |
| 551146 | Mouse anti-human CD106 | 51-10C9 | FITC | 100 tests |
| 551148 | Mouse anti-human CD106 | 51-10C9 | Cy-Chrome™ | 100 tests |
| 551138 | Mouse anti-human CD161 (NKR-P1A) | DX12 | Cy-Chrome | 100 tests |
| 551312 | Mouse anti-human c-Mpl | BAH-1 | Purified | 0.1 mg |
| 551313 | Mouse anti-human c-Mpl | BAH-1 | PE | 100 tests |
| 551314 | Mouse anti-human c-Mpl | BAH-1 | APC | 100 tests |
| 550951 | Mouse anti-human Cytokeratin 14,15,16 and 19 | KA4 | Purified | 0.1 mg |
| 551264 | Mouse anti-human DC-SIGN | DCN46 | FITC | 100 tests |
| 551265 | Mouse anti-human DC-SIGN | DCN46 | PE | 100 tests |
| 551796 | Mouse anti-human fetal hemoglobin | 2D12 | Purified | 0.1 mg |
| 551797 | Mouse anti-human fetal hemoglobin | 2D12 | PE | 100 tests |
| 551285 | Mouse anti-human HLA-A2 | BB7.2 | FITC | 0.1 mg |

| Cat No. | Description | Clone | Format | Size |
|---|---|---|---|---|
| **Human Cell Surface Reagents *(continued)*** | | | | |
| 551127 | Mouse anti-human IL-8 RB | 6C6 | APC | 100 tests |
| 551136 | Mouse anti-human mannose Receptor | 19.2 | Cy-Chrome™ | 100 tests |
| 550935 | Mouse anti-human μ-Calpain | B27D8 | Purified | 0.1 mg |
| 550936 | Mouse anti-human μ-Calpain | B27D8 | PE | 100 tests |
| 551056 | Mouse anti-human PRR2 | R2.525 | Purified | 0.1 mg |
| 551057 | Mouse anti-human PRR2 | R2.525 | PE | 100 tests |
| 551292 | Mouse anti-human TAP2 | TAP2.17 | Purified | 0.1 mg |
| 550795 | Mouse IgG1, kappa isotype control | MOPC-21 | PerCP-Cy5.5 | 0.1 mg |
| 550915 | IP30 FITC Set | | FITC | Set |
| **Ig/2nd Step Reagents** | | | | |
| 551505 | F(ab')2 Rat anti-mouse IgG (multiple adsorption) | | Biotin | 0.1 mg |
| **Immunohistochemistry Reagents** | | | | |
| 550891 | BrdU | | Buffer | 5 mg |
| 551249 | Mouse anti-human DC-SIGN | DCN46 | Purified | 1 ml |
| 551320 | Mouse anti-mouse follicular dendritic cell | FDC-M1 | Purified | 1 ml |
| 551004 | Mouse anti-human MMP-7 (Matrilysin) | ID2 | Purified | 1 ml |
| **Cytometric Bead Array Products** | | | | |
| 551809 | Human Th1/Th2 Cytokine Cytometric Bead Array (CBA) Kit - II (with IL-6) | | Kit | 50 tests |
| 551811 | Human Inflammation Cytometric Bead Array (CBA) Kit | | Kit | 50 tests |
| 551810 | Human Th1/Th2 Cytokine Standards | | lyophilized | 1 vial |
| 552124 | Human Active Caspase-3 CBA | | Kit | 100 tests |
| please inquire | Human Anaphylatoxin CBA | | Kit | 50 tests |
| **Mouse Cell Surface Reagents** | | | | |
| 552051 | Rat anti-mouse CD4 (L3T4) | GK1.5 | APC-Cy7 | 0.1 mg |
| 552094 | Rat anti-mouse CD45R/B220 | RA3-6B2 | APC-Cy7 | 0.1 mg |
| 552132 | Non-Cytolytic Mouse CTLA-4 - IgG Fusion Protein | ---- | NA/LE | 0.25 mg |
| 552133 | Non-Cytolytic Mouse CTLA-4 - IgG Fusion Protein | ---- | NA/LE | 0.5 mg |
| 551776 | Rat anti-mouse Dendritic Cells | 33D1 | Purified | 0.5 mg |
| 551771 | Mouse anti-mouse DO-11.10 Clonotypic TCR | KJ1-26 | Purified | 0.25 mg |
| 551772 | Mouse anti-mouse DO-11.10 Clonotypic TCR | KJ1-26 | PE | 0.1 mg |
| 551769 | Hamster anti-mouse H-2M3 | 130 | Purified | 0.1 mg |
| 552015 | Rat anti-mouse ICOS | 7E.17G9 | Purified | 0.5 mg |
| 551864 | Rat anti-mouse Lambda 5 | LM34 | Purified | 0.1 mg |
| 551865 | Rat anti-mouse Lambda 5 | LM34 | Biotin | 0.1 mg |
| 552093 | Rat anti-mouse Ly-6G (Gr-1) and Ly-6C | RB6-8C5 | PerCP-Cy5.5 | 0.1 mg |
| 552013 | Mouse anti-mouse Ly-49A BALB and B6 | JR9-318 | Purified | 0.1 mg |
| 552014 | Mouse anti-mouse Ly-49A BALB and B6 | JR9-318 | Biotin | 0.1 mg |
| 551891 | Hamster anti-mouse PD-1 | J43 | Purified | 0.1 mg |
| 551892 | Hamster anti-mouse PD-1 | J43 | PE | 0.1 mg |
| 551862 | Rat anti-mouse Pre-B Cell Receptor | SL156 | Purified | 0.1 mg |
| 551863 | Rat anti-mouse Pre-B Cell Receptor | SL156 | Biotin | 0.1 mg |
| **Rat Cell Surface Reagents** | | | | |
| 551450 | Mouse anti-rat CD5 | HIS47 | Purified | 0.1 mg |
| 551449 | Mouse anti-rat CD5 | OX-19 | FITC | 0.1 mg |
| 551402 | Mouse anti-rat CD45RA | OX-33 | PE | 0.1 mg |
| 551451 | Mouse anti-rat CD45RC | OX-22 | Purified | 0.1 mg |
| 552011 | Mouse anti-rat CD48 | OX-45 | Purified | 0.1 mg |
| 551452 | Mouse anti-rat CD53 | OX-44 | Purified | 0.1 mg |
| 551398 | Hamster anti-rat CD62L (L-selectin, LECAM-1) | HRL1 | PE | 0.1 mg |
| 551458 | Mouse anti-rat CD63 (ME491) | AD1 | Purified | 0.1 mg |
| 551396 | Mouse anti-rat CD86 (B7-2) | 24F | PE | 0.1 mg |
| 551401 | Mouse anti-rat CD90 (Thy-1) | OX-7 | PE | 0.1 mg |

# NEW Products from BD Biosciences *(continued from page 33)*

| Cat No. | Description | Clone | Format | Size |
|---|---|---|---|---|
| **Rat Cell Surface Reagents *(continued)*** | | | | |
| 551469 | Mouse anti-rat High Affinity IgE receptor (FcεRI) | BC4 | Purified | 0.1 mg |
| 551770 | Mouse anti-rat Mast Cells | AR32AA4 | Purified | 0.1 mg |
| 552012 | Mouse anti-rat CD147 | OX-47 | Purified | 0.1 mg |
| **Pig Cell Surface Reagents** | | | | |
| 551543 | Rat anti-pig γδ T Lymphocytes | MAC320 | Purified | 0.1 mg |
| 551541 | Mouse anti-pig CD5 | b53b7 | Purified | 0.1 mg |
| 551507 | Mouse anti-pig CD6 | a38b2 | Purified | 0.1 mg |
| 551508 | Mouse anti-pig CD11a (Integrin αL chain) | BL2F1 | Purified | 0.1 mg |
| please inquire | Mouse anti-pig CD11b (Integrin αM chain) | 2F4/11 | Purified | 0.1 mg |
| 551542 | Rat anti-pig CD44H (Pgp-1, Hermes antigen) | MAC329 | Purified | 0.1 mg |
| please inquire | Mouse anti-pig CD45 (Leukocyte Common Antigen) | 2A5 | Purified | 0.1 mg |
| 551536 | Mouse anti-pig CD45RC | 3a56 | Purified | 0.1 mg |
| please inquire | Mouse anti-pig CD46 | JM6C11 | Purified | 0.1 mg |
| 551544 | Mouse anti-pig CD235a (Glycophorin A) | 1AC11 | Purified | 0.1 mg |
| 551537 | Mouse anti-pig SLA-DR | 1F12 | Purified | 0.1 mg |
| 551538 | Mouse anti-pig SLA-DQ | BL4H2 | Purified | 0.1 mg |
| **Molecular Biology Reagents - BD RiboQuant™ Multi-Probe Template Sets** | | | | |
| **Mouse Cell Cycle Regulator/Complement** | | | | |
| 551490 | mComplement RiboQuant Multi-Probe Template Set | | | 10 reactions |
| **Mouse Angiogenesis** | | | | |
| 551418 | mAngio-1 RiboQuant Multi-Probe Template Set | | | 10 reactions |
| **Mouse Matrix Metalloproteinase** | | | | |
| 551276 | mMMP-1 RiboQuant Multi-Probe Template Set | | | 10 reactions |
| 550249 | mMMP-2 RiboQuant Multi-Probe Template Set | | | 10 reactions |
| **Mouse Developmental Gene** | | | | |
| 550925 | mWnt-1 RiboQuant Multi-Probe Template Set | | | 10 reactions |
| 550249 | mWnt-2 RiboQuant Multi-Probe Template Set | | | 10 reactions |
| **Human Cytokines and Chemokines** | | | | |
| 551787 | hCK-8 RiboQuant Multi-Probe Template Set | | | 10 reactions |
| 551488 | hCK-9 RiboQuant Multi-Probe Template Set | | | 10 reactions |
| **Human DNA Repair Pathway** | | | | |
| 550251 | hDisR RiboQuant Multi-Probe Template Set | | | 10 reactions |
| **Human Matrix Metalloproteinase** | | | | |
| 551274 | hMMP-1 RiboQuant Multi-Probe Template Set | | | 10 reactions |
| 551275 | hMMP-2 RiboQuant Multi-Probe Template Set | | | 10 reactions |
| **Human Sulfotransferase** | | | | |
| 550793 | hTOX-1b RiboQuant Multi-Probe Template Set | | | 10 reactions |
| 550794 | 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*

**BD Cytometric Bead Array System**

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BD Biosciences Pharmingen has added a new twist to counting with beads. We recognize the value of small, precious samples and data reproducibility so we've developed a technology to ensure just those things.

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- avoid artifacts associated with enzyme dependent signal generation
- achieve quantitative results with less time and labor
- use a complete system with ready-to-use kits and analysis software

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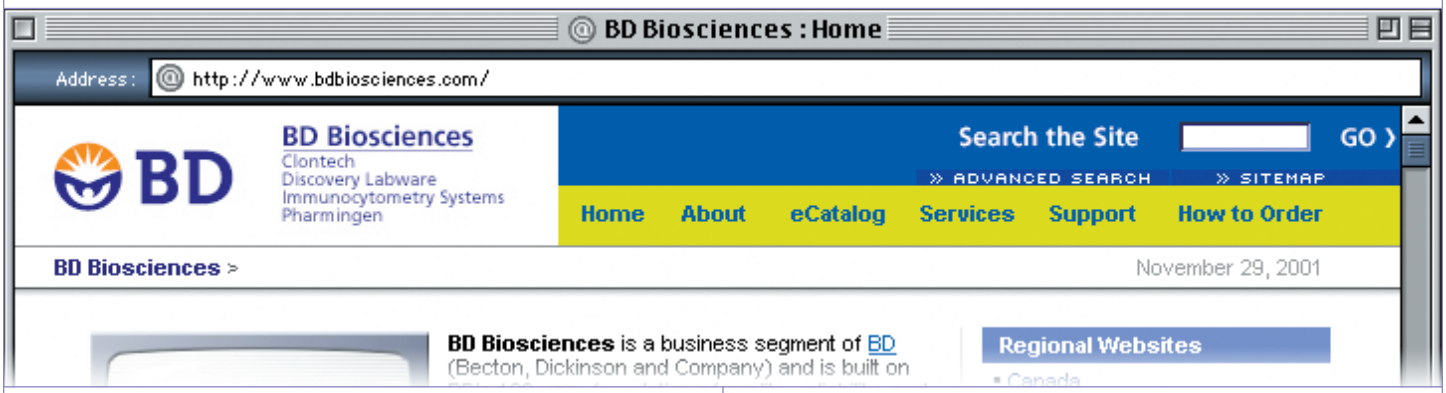
## Where We'll Be in Winter 2001/2002

| When | What | Where |
|---|---|---|
| 2001 | | |
| **December 6** | University of Pennsylvania, Vendor Show | Philadelphia, PA |
| **December 7** | Georgetown University, Vendor Show | Washington, DC |
| **December 7 - 11** | American Society of Hematology (ASH) | Orlando, FL |
| **December 8 - 12** | American Society for Cell Biology (ASCB) 2001 | Washington, DC |
| 2002 | | |
| **January 10** | University of California, San Francisco, Vendor Show | San Francisco, CA |
| **January 30** | University of Texas, Vendor Show | Houston, TX |
| **February 25 - 28** | Baculovirus & Insect Cell Culture | Santa Fe, NM |
| **March 17 - 21** | SOT - Society of Toxicology | Nashville, TN |

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Edited by Dara Grantham Wright; Designed by Azita Zarrabi



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01-81008-33A1