The BD Cytometric Bead Array System

BD Biosciences
Clontech
Discovery Labware
Immunocytometry Systems
Pharmingen

BD

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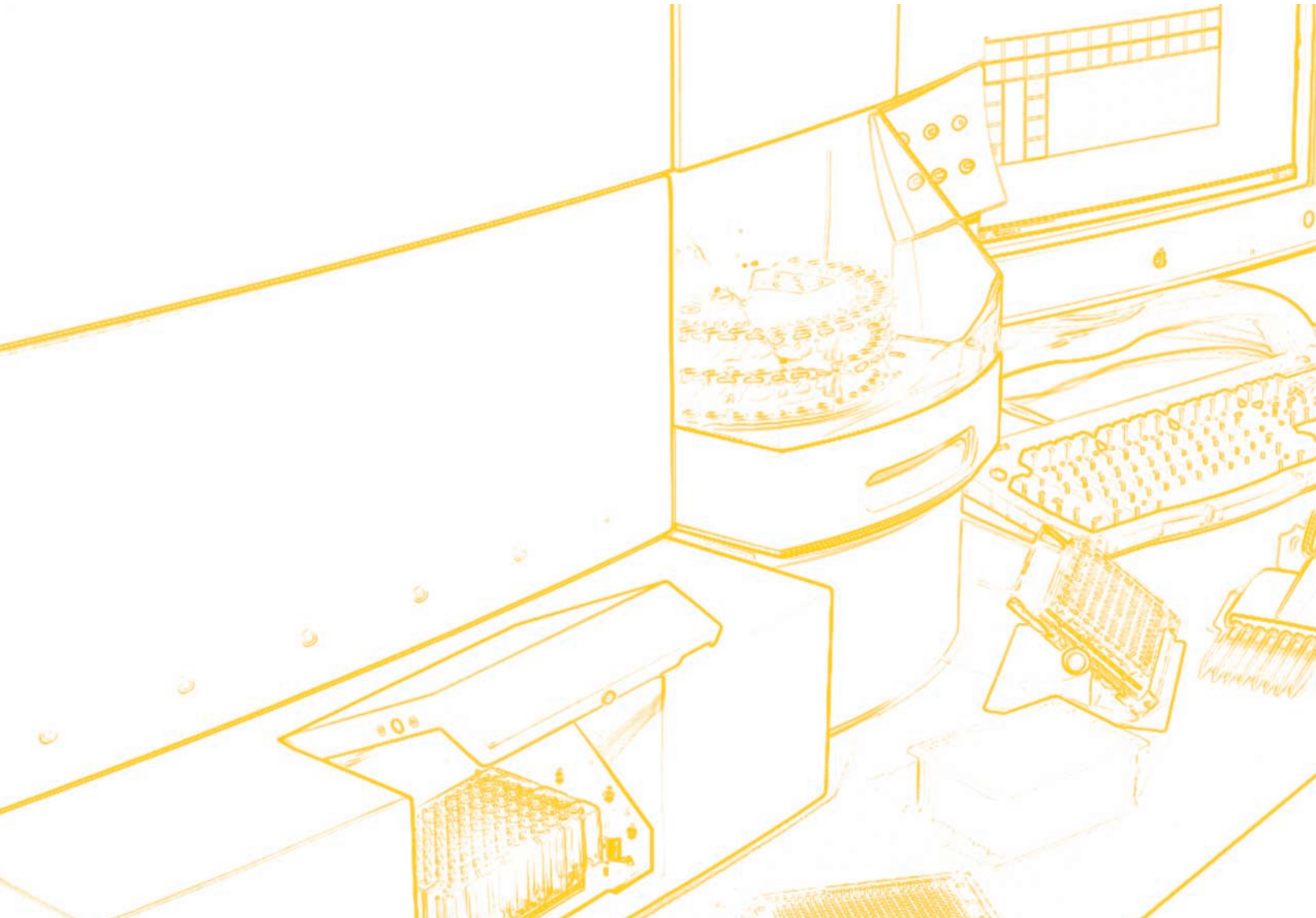
Introduction

A Multiplex Bead System You Can Count On

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 both. The innovative Cytometric Bead Array (CBA) technology allows for quantitative detection of multiple analytes in a single sample. The BD CBA System of assay kits, flow cytometers, and easy-to-use software provides reproducible data and reliable performance that you can count on time and time again.

With the BD CBA System you can:

- Get more results from a single small volume sample
- Run one standard mixture to generate standard curves for all your analytes
- Avoid artifacts associated with enzyme dependent signal generation
- Achieve quantitative results with less time and labor
- Combine versatile flow cytometers with ready-to-use kits and analysis software
- Automate sample acquisition with the BD Multiwell™ AutoSampler for increased sample throughput

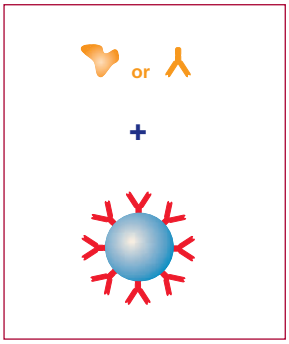


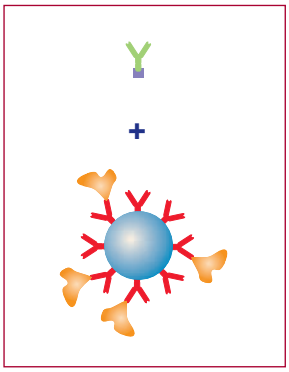
BD CBA Assay Overview

Flow cytometry is an analytical tool that allows for the discrimination of different particles on the basis of size and color. The BD CBA employs a series of particles with discrete fluorescence intensities to simultaneously detect multiple soluble analytes from a single serum, plasma, or tissue culture supernatant sample. The BD CBA, combined with flow cytometry, creates a powerful multiple analyte (multiplex) assay system.

The BD CBA system uses the sensitivity of amplified fluorescence detection by flow cytometry to measure soluble analytes in a particle-based immunoassay. The combined advantages of the broad dynamic range of fluorescence detection via flow cytometry, and the efficient capturing of analytes via suspended particles coated with distinct capture antibodies enable the BD CBA to use fewer sample dilutions to determine analyte concentration in substantially less time (compared to conventional ELISA).

The specific capture beads are mixed with the phycoerythrin-conjugated detection antibodies and then incubated with recombinant protein standards or test samples to form sandwich complexes. Following acquisition of sample data using the flow cytometer, the sample results are generated in graphical and tabular format using the BD CBA Analysis Software.





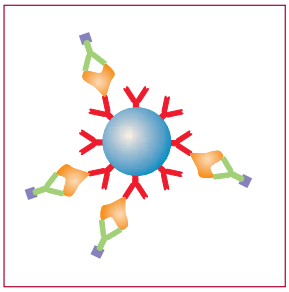
INCUBATE

WASH

Typical BD CBA Assay Protocol

1. Add unknown(s) or standards to capture bead array.
2. Add detection reagents and incubate.
3. Wash and acquire samples.
4. Perform batch analysis using BD CBA Software.

Note: Single analyte shown. Always refer to kit protocol for specific instructions.



ACQUIRE

ANALYZE

Performance Characteristics & Representative Data

All BD CBA Kits are rigorously tested for performance characteristics including sensitivity, spike recovery, dilution linearity, specificity, intra- and inter-assay precision.

Representative data are shown below in Tables 1-6.

Intra- and Inter-assay Precision

Cytokine	Intra-assay Avg. (%)	Inter-assay Avg. (%)
IFN-γ	3.7	8
TNF-α	4	6.7
IL-10	2.3	5.7
IL-5	4.7	6.7
IL-4	4	5.3
IL-2	3	7.3

Table 1. Replicate samples were analyzed with the Human Th1/Th2 Cytokine CBA Kit and the average CV% of the calculated cytokine values (80 pg-2500 pg/ml) were determined.

Cytokine	Intra-assay Avg. (%)	Inter-assay Avg. (%)
IL-12p70	3.7	7.3
TNF-α	8.3	12
IL-10	5.7	9.3
IL-6	6.3	9
IL-1β	5.7	10
IL-8	3.7	5

Table 2. Replicate samples were analyzed with the Human Inflammation CBA Kit and the average CV% of the calculated cytokine values (80 pg-2500 pg/ml) were determined.

Cytokine	Intra-assay Avg. (%)	Inter-assay Avg. (%)
TNF-α	6.7	11.3
IFN-γ	3.3	7.7
IL-5	6	12.3
IL-4	4.7	9.3
IL-2	5	8.3

Table 3. Replicate samples were analyzed with the Mouse Th1/Th2 Cytokine CBA Kit and the average CV% of the calculated cytokine values (80 pg-2500 pg/ml) were determined.

Cytokine	Intra-assay Avg. (%)	Inter-assay Avg. (%)
IFN-γ	3.3	7.3
TNF-α	3	4
IL-10	2.7	4.7
IL-6	2.7	4.3
IL-4	3.3	4.3
IL-2	3.7	6.3

Table 4. Replicate samples were analyzed with the Human Th1/Th2 Cytokine CBA Kit - II and the average CV% of the calculated cytokine values (80 pg-2500 pg/ml) were determined.

Cytokine	Intra-assay Avg. (%)	Inter-assay Avg. (%)
Active Caspase-3	8.7	11.5

Table 5. Replicate samples were analyzed with the Human Active Caspase-3 CBA Kit and the average CV% of the calculated protein values (94 - 3000 Units/ml) were determined.

Spike Linearity

Sample Matrix		Cytokine Measured					
		IFN-γ	TNF-α	IL-10	IL-5	IL-4	IL-2
Culture Media	slope	1.03	1.042	0.943	0.994	0.975	0.983
	r^2	0.999	0.996	0.999	0.999	0.999	0.993
Plasma	slope	0.978	0.839	0.877	0.865	0.88	0.815
	r^2	0.993	0.998	0.998	0.996	0.983	0.995
Serum	slope	0.949	0.885	0.885	0.891	0.865	0.886
	r^2	0.994	0.999	0.995	0.995	0.996	0.986

n = 4 reps. 5 donor pools for biologicals

Table 6. In two experiments, the above matrices were spiked with human recombinant IL-2, IL-4, IL-5, IL-10, TNF-α, and IFN-γ, then serially diluted with Assay Diluent. The spiked dilution samples were analyzed using the Human Th1/Th2 Cytokine CBA and the resulting dilution curve was plotted for each matrix. The resulting slopes and correlation coefficients (r^2) are summarized.

Human Active Caspase-3 CBA Kit

A **NEW** Addition to the BD CBA Family of Products

The Human Active Caspase-3 Cytometric Bead Array (CBA) Kit is a single bead assay for the rapid quantification of active caspase-3 in cell lysates by flow cytometry and represents a breakthrough in the application of flow cytometry tools for the field of Cell Biology.

The presence of active caspase-3 is an indication that cells are undergoing apoptosis. The Human Active Caspase-3 CBA Kit contains a lyophilized apoptotic cell lysate for generating active caspase-3 standard curves. This enables relative quantification of active caspase-3 in the researcher's cell lysate samples (Figure 1). The Human Active Caspase-3 CBA Kit offers an alternative to both conventional immunoprecipitation and western blot assays and is capable of measuring active caspase-3 over a two-log dynamic range using a simple 4-hour assay protocol. The CBA technology has comparable analytical sensitivity and a wider dynamic range than conventional ELISA.

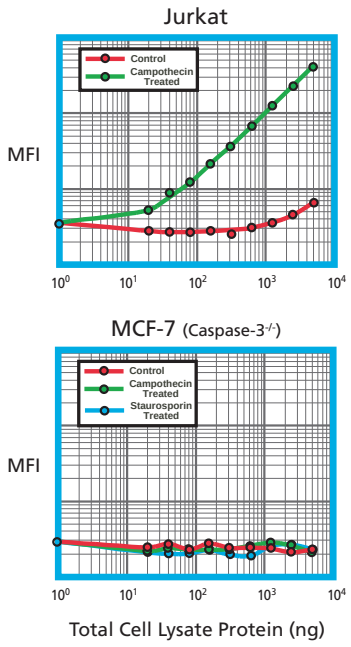


Figure 1. BD CBA analysis of Active Caspase-3.

Detection of Non-Human Primate Cytokine Levels

Did You Know That The Human BD CBA Kits Also Measure Some Non-Human Primate Cytokines?

Many BD CBA customers have determined that they are able to detect positive signals for rhesus and cynomolgus macaque samples when using the Human Th1/Th2 Cytokine CBA Kit. The BD CBA results have been confirmed by ELISA using the BD CBA antibody pairs with activated cell culture samples from both rhesus and cynomolgus macaques. The cross-reactivity of BD CBA Human assays with non-human primate (NHP) analytes have not yet been normalized to native or recombinant NHP proteins, so direct quantitation is not yet available (Table 7, Figure 2).

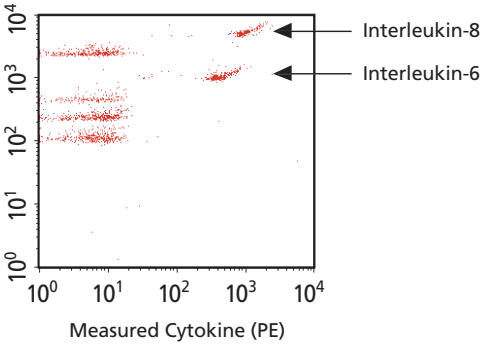


Figure 2. Representative Non-Human Primate Reactivity. Cynomolgus macaque serum tested using the Human Inflammation CBA Kit.

Table 7. BD CBA Cross-Reactivity with Non-Human Primate Analytes.

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

Standardization of the Human Th1/Th2 BD CBA Cytokine Standards to International (NIBSC) Standards

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. 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 8), 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. 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. 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 8. As shown in **Figure 3**, 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.

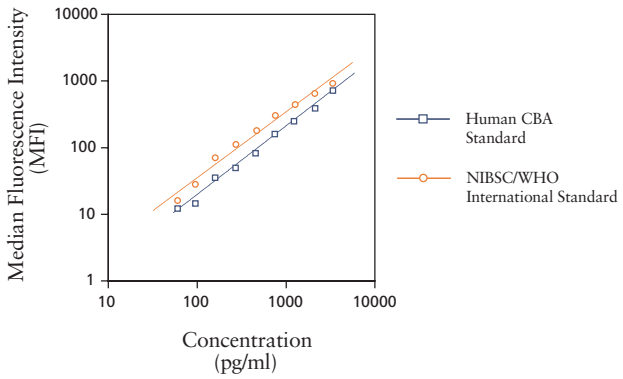


Figure 3. Human IFN-γ titration curves.

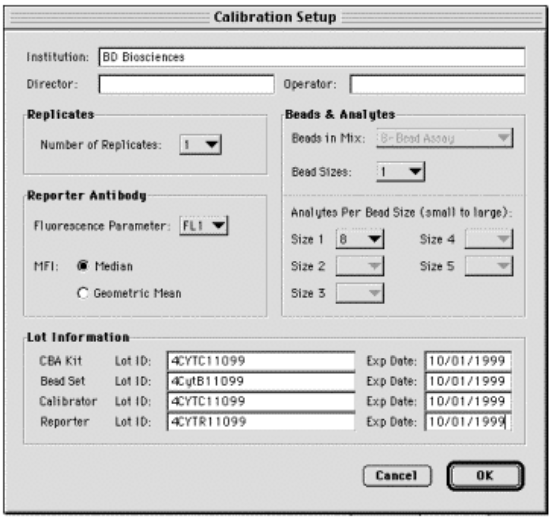
Table 8. Conversion Factors Between the BD CBA Human Th1/Th2 Cytokine Standards and the NIBSC Standards.

	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

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.

BD CBA Software

The CBA analysis software is a powerful and flexible tool built as an Add-In for Microsoft Excel 5, 95, 2000, and 2001 on the Macintosh Power PC, G3, or G4 Computer or Excel 2000 for Microsoft Windows (Table 9). Accommodating multiple sizes and intensities of particles, it provides a foundation for bead array analyses in the research environment. A wide variety of preset configurations enable the user to set up standard dilution series, generate calibration curves using a 4-parameter logistic curve fit model, and subsequently compare unknowns. Appropriate reports can be generated from each step in the process. The analysis of BD CBA data is optimized when using the BD CBA Software. The software should be installed according to the instructions in the Software User's Guide. The Software User's Guide and latest software updates can be downloaded at **http://www.bdbiosciences.com/pharmingen/CBA/**.



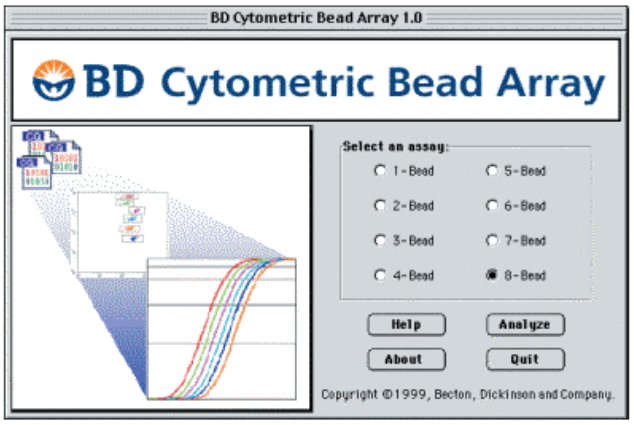


Table 9. BD CBA Software Compatibility

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

BD CBA Troubleshooting Tips

Care in Mixing

Inadequate mixing can lead to little or no beads in the assay readout. We recommend vortexing beads immediately before mixing with standards or samples and again before analysis using the flow cytometer. Vortex each sample tube for 3-5 seconds before placing the tube on the flow cytometer. This will yield better discrimination of the bead populations in the FL3 channel.

Debris or non-bead events? Optimizing the instrument settings.

When detecting debris (FSC/SSC) during sample acquisition, increase the FSC threshold or further wash or dilute samples. If the problem persists after adjusting the FSC threshold, then repeat the sample wash step. If debris is still a problem, then reset the threshold using SSC and adjust as necessary.

Bead populations appear to overlap during acquisition? This may occur in samples with very high cytokine concentrations. Ensure that compensation settings have been optimized using the Cytometer Setup Beads.

Samples have little or no FL2 fluorescence? Check sample dilution, do not alter recommended assay incubation time, and protect sample tubes from light during the incubation step.

High background fluorescence or all samples brightly positive?

High background could be due to the sample being highly concentrated. Test various sample dilutions. If all samples are positive or above the top standard's median fluorescence intensity, dilute samples further as the samples may be too concentrated.

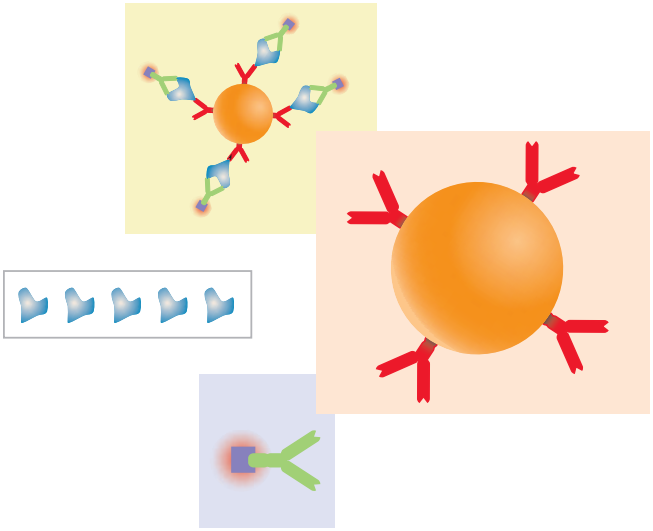
Must a new vial of standard be used with each experiment?

Once reconstituted, do not use standards after 12 hours. Use a fresh standard dilution set for each experiment.

Other Helpful Hints

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

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



BD Multiwell AutoSampler

Now you can increase your throughput and decrease your hands-on time with the new BD Multiwell™ AutoSampler for use with BD Biosciences flow cytometers.

Product Highlights

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

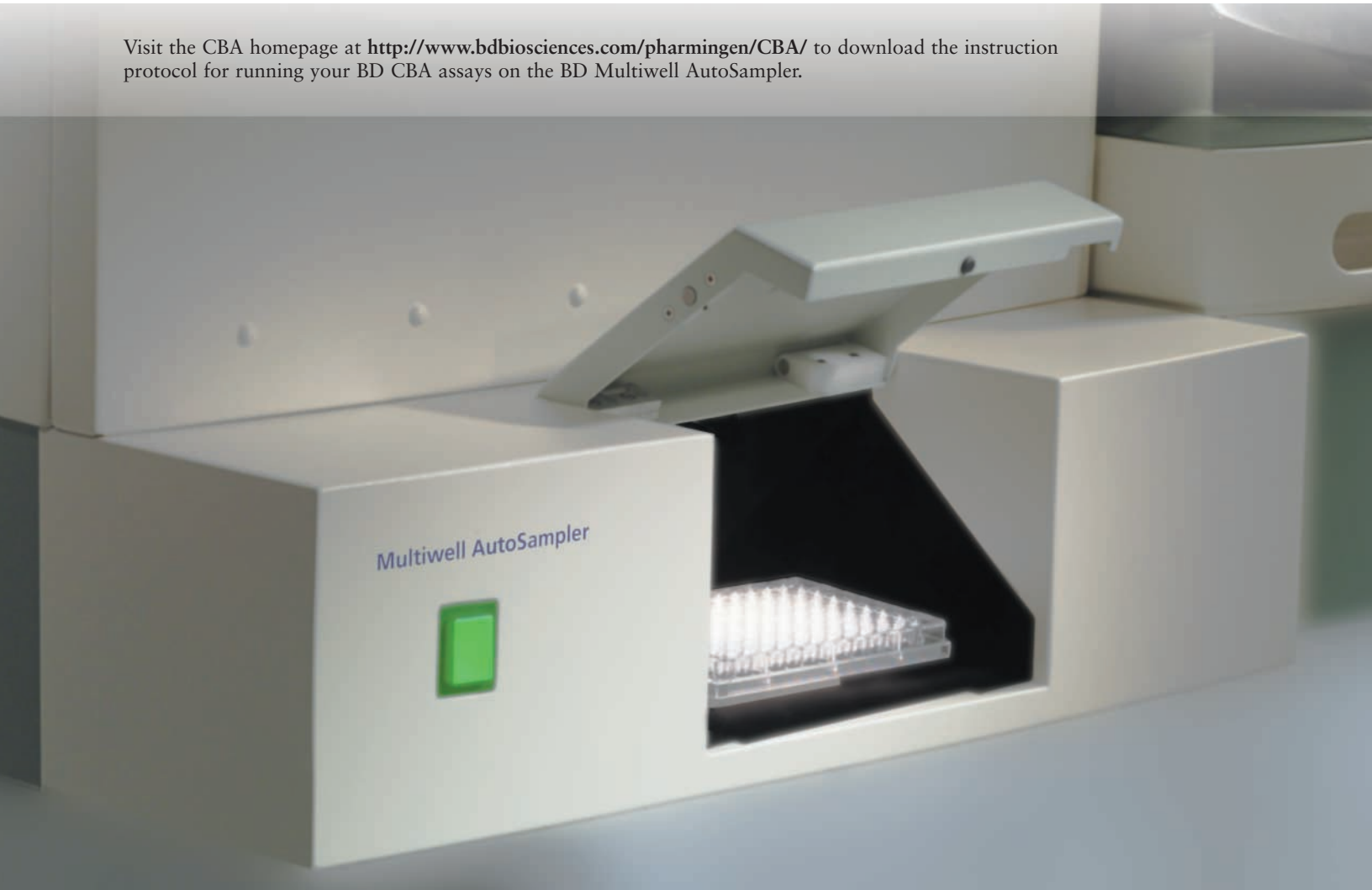
Features

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

Specifications

- Mixing: selectable for 0–3 mix repetitions, definable from 10–250 µL
- Washing: selectable for 1–3 wash repetitions
- Sample volume: definable from 25–250 µL
- Sample carryover: <1% with one wash cycle between cell samples
- Bench space: no additional bench space required

Visit the CBA homepage at **http://www.bdbiosciences.com/pharmingen/CBA/** to download the instruction protocol for running your BD CBA assays on the BD Multiwell AutoSampler.



BD CBA Products Available from BD Biosciences Pharmingen

	Cat. No.	Description	Analytes	Format	Size
Human Kits					
	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 , and IL-12p70	Kit	50 tests
NEW	552124	Human Active Caspase-3 CBA Kit	Active Caspase-3	Kit	100 tests
NEW	*Avail. May 2002*	Human Anaphylatoxin CBA Kit	C3a, C4a, C5a	Kit	50 tests
Mouse Kits					
	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
NEW	*Avail. June 2002*	Mouse Inflammation CBA Kit	IL-1β , IL-6, IL-10, TNF-α , IL-12p70, and MCP-1	Kit	50 tests
Other					
NEW	551810	Human Th1/Th2 Cytokine Standards	IL-2, IL-4, IL-5, IL-6, IL-10, TNF-α , IFN-γ	lyophilized	1 vial
	550065	BD CBA Software	Mac and PC Compatible CD-Rom and User's guide	CD	1 CD
NEW		BD Multiwell AutoSampler	*Please contact your BD Biosciences Sales Representative for pricing details*		

Custom Services

Need bulk sizing or to mix-and-match the specificities listed above in the BD CBA product list? We can help. Our Custom Products and Services Team at BD Biosciences Pharmingen can customize a kit using currently available specificities. Our Custom Products and Services Team can also test your samples using the BD CBA technology and provide you with the data. Please ask your local Sales Representative for pricing.

Special Offers

Need a new BD Flow Cytometer? Please contact your local Sales Representative about BD CBA reagent rental opportunities.

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BD Biosciences

Clontech
Discovery Labware
Immunocytometry Systems
Pharmingen

Asia Pacific
BD Singapore
Tel 65.6861.0633
Fax 65.6860.1590

Japan
Nippon Becton Dickinson
Tel 81.24.593.5405
Fax 81.24.593.5761

Clontech Company
(Clontech Products)
Tel 81.3.5324.9609
Fax 81.3.5324.9637

Canada
BD Biosciences
Toll free 888.259.0187
Tel 905.542.8028
Fax 905.542.9391
canada@bd.com

Europe
Belgium
Tel 32.53.720.211
Fax 32.53.720.450

United States
BD Biosciences
Clontech
Fax 650.354.0775
Discovery Labware
Fax 978.901.7493
Immunocytometry Systems
Fax 408.954.2347
Pharmingen
Fax 858.812.8888
Customer/Technical Service
Toll free 877.232.8995
www.bdbiosciences.com

Argentina/Paraguay/Uruguay
Tel 54.11.4551.7100
Fax 54.11.4551.7400

Australia/New Zealand
Australia
Tel 61.2.8875.7000
Fax 61.2.8875.7200
bd_anz@bd.com

New Zealand
Tel 64.9.574.2468
Fax 64.9.574.2469
bd_anz@bd.com

Austria
Scientific Support
Tel 43.1.706.36.60.44
Fax 43.1.706.36.60.45
BDBsupport_GSA@europe.bd.com
Customer Service
Tel 43.1.706.36.60
Fax 43.1.706.36.60.11

Belgium
Tel 32.53.720.600
Fax 32.53.720.220
technical_support_bdbelgium@europe.bd.com
Customer Service
Tel 32.53.720.550
Fax 32.53.720.549
customer_service_bdbelgium@europe.bd.com

Brazil
Tel 55.11.5185.9995
Fax 55.11.5185.9895

Central America/Caribbean
Tel/Fax 506.290.7318

Chile
Tel 56.2 460.0380 x21
Fax 56.2 460.0306

China
Tel 8610.6418.1608
Fax 8610.6418.1610

Colombia
Tel 57.1.345.0510
Fax 57.1.255.6320

Denmark
Tel 45.43.43.45.66
Fax 45.43.43.41.66
bdbsupport_dk@europe.bd.com

East Africa
Tel 254.2.341157
Fax 254.2.341161
bd@africaonline.co.ke

Eastern Europe
Tel 49.6221.305.161
Fax 49.6221.305.418

Egypt
Tel 202.268.0181
Fax 202.266.7562

Finland
Tel 358.9.88.70.7832
Fax 358.9.88.70.7817
bdbsupport_fi@europe.bd.com

France
Tel 33.4.76.68.36.36
Fax 33.4.76.68.35.06
Scientific Support
Tel 33.4.76.68.34.25
Fax 33.4.76.68.55.71
Customer Service
Tel 33.4.76.68.37.32
Fax 33.4.76.68.35.06

Germany
Scientific Support
Tel 49.6221.305.525
Fax 49.6221.305.530
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Fax 49.6221.303.609
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Fax 971.4.337.95.51

The Netherlands
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Fax 31.20.582.94.26
technical_support_bdholland@europe.bd.com
Customer Service
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Fax 31.20.582.94.21
customer_service_bdholland@europe.bd.com

Norway
Immunocytometry Systems & Pharmingen
Laborel S/A
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South Africa
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Fax 27.11.807.19 53

Spain
Immunocytometry Systems & Pharmingen
Scientific Support
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Fax 34.91.848.81.04
Clontech
Tel 34.91.848.81.85
Fax 34.91.848.81.04

Sweden
Tel 46.08.775.51.10
Fax 46.08.775.51.11
bdbsupport_se@europe.bd.com

Switzerland
Scientific Support
Tel 41.61.485.22.91
Fax 41.61.485.22.92
BDBsupport_GSA@europe.bd.com
Customer Service
Tel 41.61.485.22.22
Fax 41.61.485.22.00
customerservice.bdb.ch@europe.bd.com

Taiwan
Tel 8862.2722.5660
Fax 8862.2725.1768

Thailand
Tel 662.643.1371
Fax 662.643.1381

Turkey
Tel 90.212.222.87.77
Fax 90.212.222.87.76

UK
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Fax 44.1865.78.16.27

Uruguay
Clontech
Tel 598.2.480.8550
Fax 598.2.481.3313

Venezuela
Tel 58.212.472.3736
Tel/Fax 58.212.442.4477

West Africa
Sobidis
Tel 225.20.33.40.32
Fax 225.20.33.40.28