The BD™ Cytometric Bead Array System (CBA)

BD Biosciences
Clontech
Discovery Labware
Immunocytometry Systems
Pharmingen

BD

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Introduction

A Multiplex Bead System You Can Count On

BD Biosciences has added a new twist to counting with beads. We recognize the value of precious samples and data reproducibility, so we've developed a technology to ensure both. The innovative BD™ Cytometric Bead Array (CBA) technology allows for quantitative detection of multiple analytes in a single serum, plasma, tissue culture supernatant, or cell lysate 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™ Cytometric Bead Array (CBA) System You Can:

- Get multiple 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 and increase throughput with the plate-based BD FACSArray bioanalyzer or other BD flow cytometers equipped with the high throughput sampler (HTS) option.

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 with 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 (PE) 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.

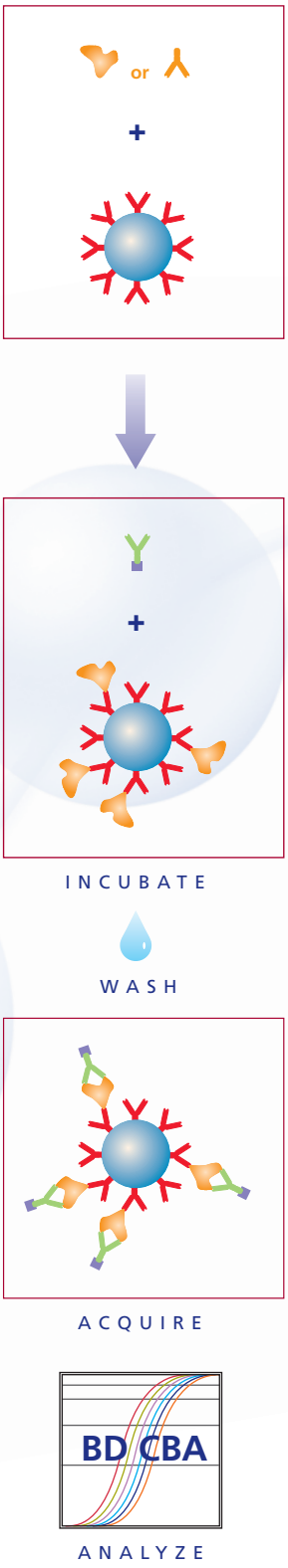

Figure 1. Typical BD CBA assay protocol. *A single analyte is shown for representational purposes.*

Quality is Built into Every BD™ CBA Product

The powerful BD™ Cytometric Bead Array (CBA) assays enable multiplex analysis of complex biological samples on a flow cytometer. Each assay has been stringently developed for ease-of-use, broad instrument compatibility and rapid data analysis, sensitivity, reproducibility, and quality.

As with any complex assay, the ability of the novice user to become comfortable with the assay is critical. In the BD CBA system, ease-of-use has been engineered into each and every kit. The standards for each multiplex kit are provided together in a lyophilized format that yields a ready-to-use stock standard solution upon reconstitution. Each kit includes a pre-diluted detection reagent containing each detector antibody formulated at their optimal concentration. Even the assay protocol has been designed to limit handling steps (Figure 1) and hands-on time by allowing the researcher to add all kit reagents, samples, and/or standards to their assay well or tube at the same time. The assay is incubated and then completed with a short wash step followed by sample analysis on a flow cytometer.

Every BD CBA assay is compatible with any flow cytometer that is equipped with a 488 nm laser and capable of detecting and distinguishing fluorescence emissions at 576 and 670 nm. In addition, analysis software capable of saving data in an FCS 2.0 file format is required. This includes all of the BD flow cytometry systems, however, it should be noted that stream-in-air instruments will yield lower assay sensitivities than other instruments. The data collected on a flow cytometer, once saved as an FCS 2.0 file, can be rapidly analyzed using the BD CBA Software. The BD CBA Software enables linear regression analysis of data files and extrapolation of sample values by comparison against a known standard curve. The software also has broad compatibility as it is offered in both PC and Mac-compatible formats, requiring only Microsoft Excel® to run. With the BD CBA Software, sample results are obtained within minutes of completing an experiment.

Considerable effort has been made to ensure that each BD CBA kit has the highest possible sensitivity and best reproducibility. Each antibody pair used in the kits is evaluated for dynamic range, sensitivity, and parallel titration curves to native biological samples (Figures 2 and 3). In addition, the scientists at BD Biosciences have formulated the assay diluent and wash buffers in each kit to reduce detrimental effects of serum and plasma proteins on assay performance. While this does not always yield comparable recovery results as single assays (eg, ELISA), it does provide quality multiplex performance.

Possibly the most important aspect of the BD CBA kits is the quality performance that is built into each one. Using techniques and methods developed at BD Biosciences, a worldwide leader in the production and conjugation of antibodies, the reagents in a BD CBA kit are stringently tested during the development process. Each capture antibody used for a capture bead has been tested both before and after it is coupled to the bead. Further, it is tested again when it is combined with the other kit reagents to complete the manufacture of a batch of kits. The same is true of the detection antibodies, which are additionally tested before and after conjugated with R-phycoerythrin (R-PE) and again when they are formulated into a detection reagent. Finally, they are tested with the other reagents in a given kit batch after they are bottled. All of this effort is made to ensure that the kit that is delivered to the researcher meets their expectations and provides equal performance every time. Further, the quality of the BD CBA kits does not end with the release of the product. We are continually looking for methods to enhance kit performance through improved antibody pairs, new standards formulations and optimized buffers for serum samples.

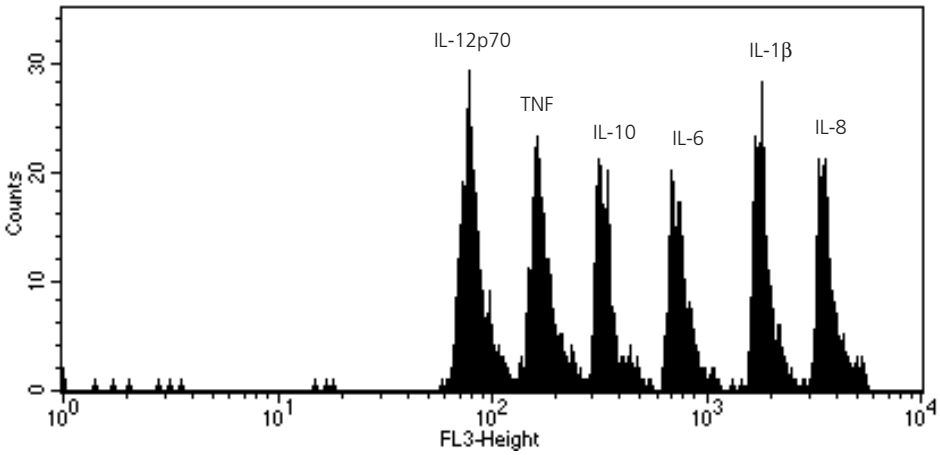

Figure 2a. Representative data generated using the BD CBA Human Inflammation Kit. Relative bead fluorescence intensities.

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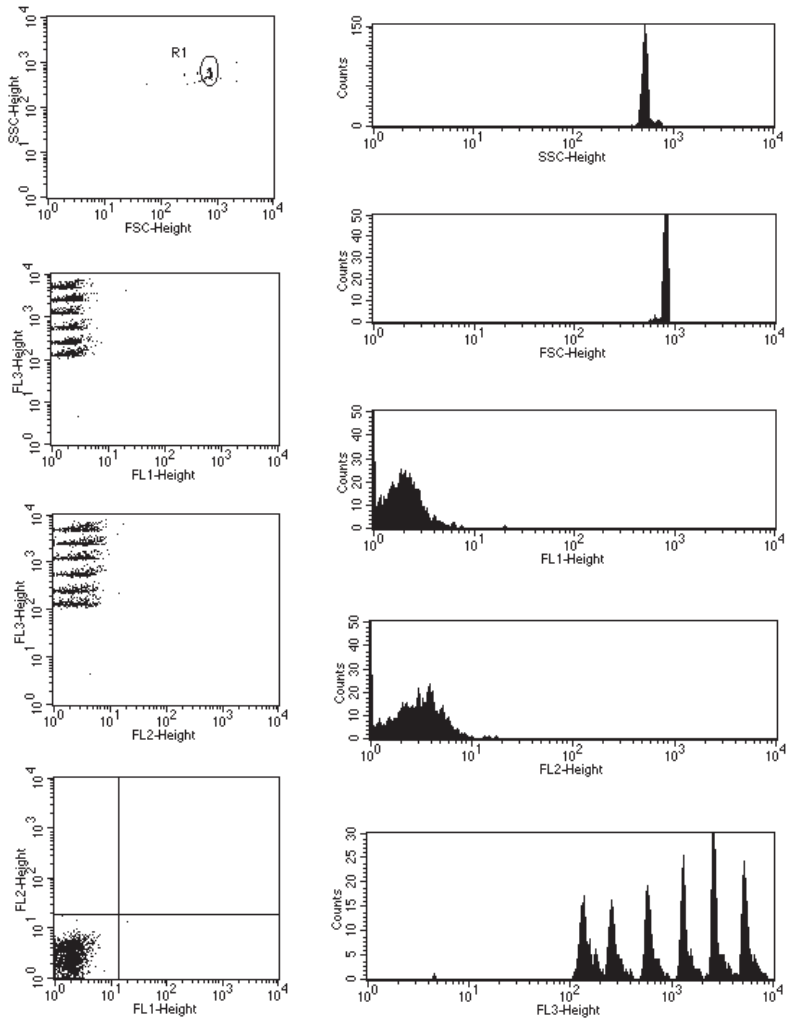
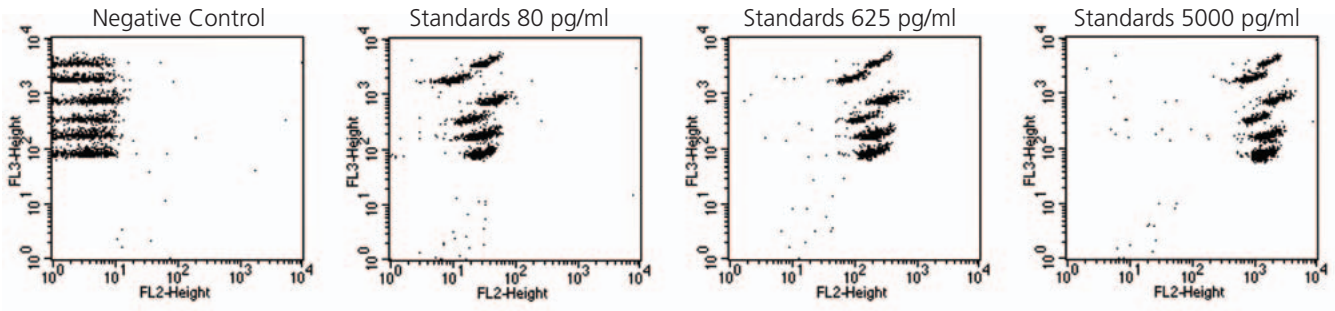
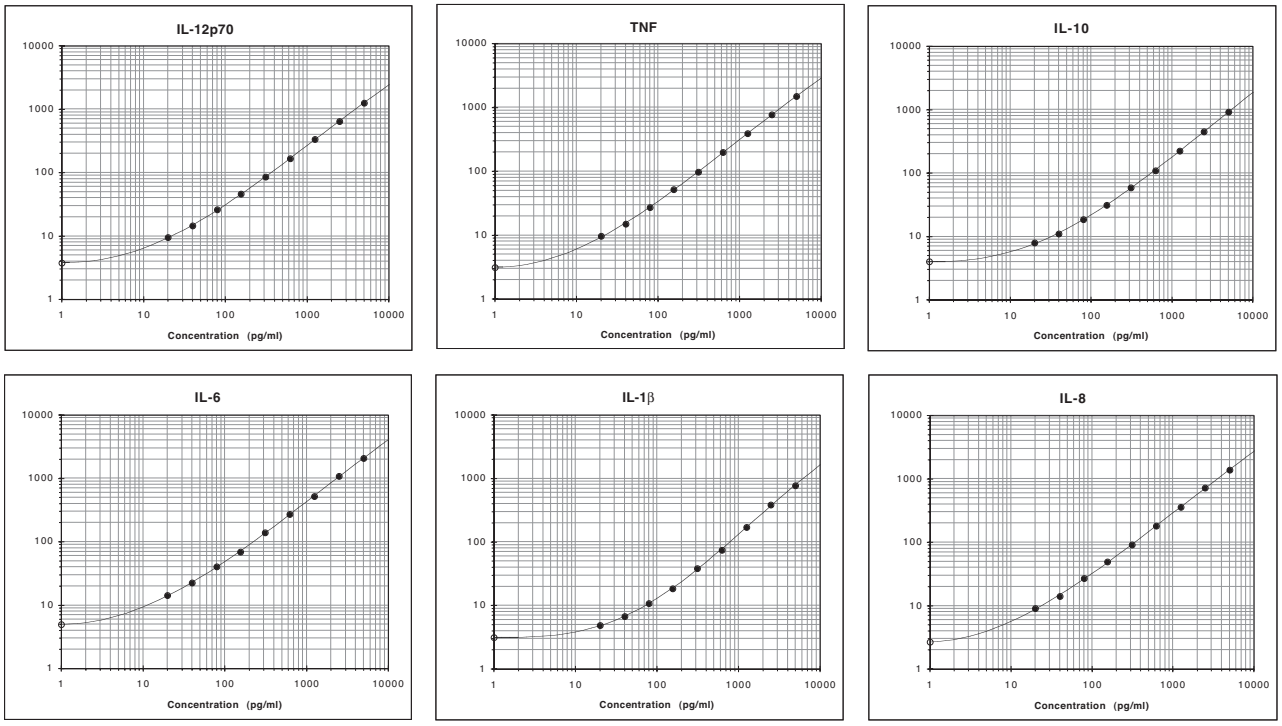


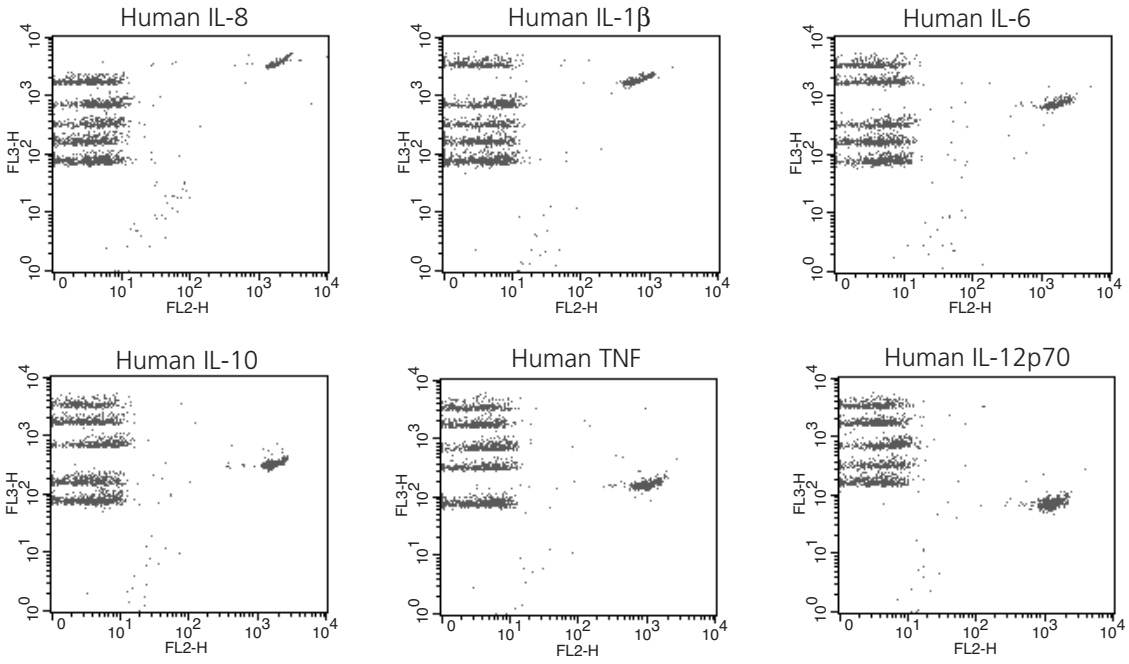
Figures 2b. Representative data generated using the BD CBA Human Inflammation Kit.
Sample data acquired using the BD CBA analysis template.



Figures 2c. Representative data generated using the BD CBA Human Inflammation Kit.
Typical standard titration data.



Figures 2d. Representative data generated using the BD CBA Human Inflammation Kit.
Sample standard curves generated with the BD CBA software.



Figures 2e. Representative data generated using the BD CBA Human Inflammation Kit.
BD CellQuest™ data for the detection of individual proteins demonstrating assay specificity.

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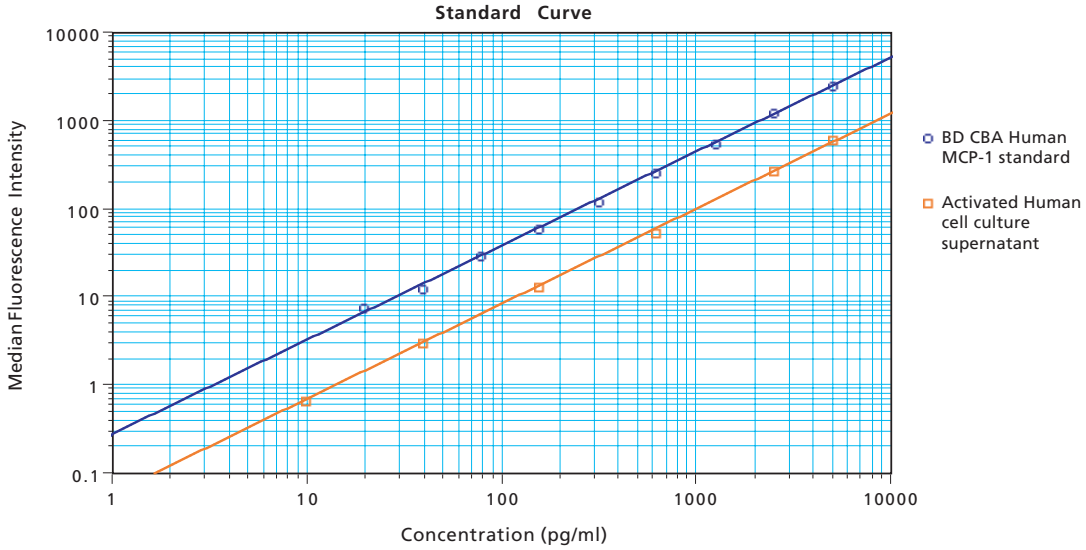

Figure 3. Human Chemokine Kit II data for MCP-1. *Representative data showing the parallel titration curves of the BD CBA Human MCP-1 standard (20-5000 pg/ml) and an activated human cell culture supernatant. For presentation purposes, the first dilution of the supernatant, 1:2, was plotted at 5000 pg/ml. Each point thereafter (1:4, 1:16, 1:64, 1:256, and 1:1024) were similarly plotted.*

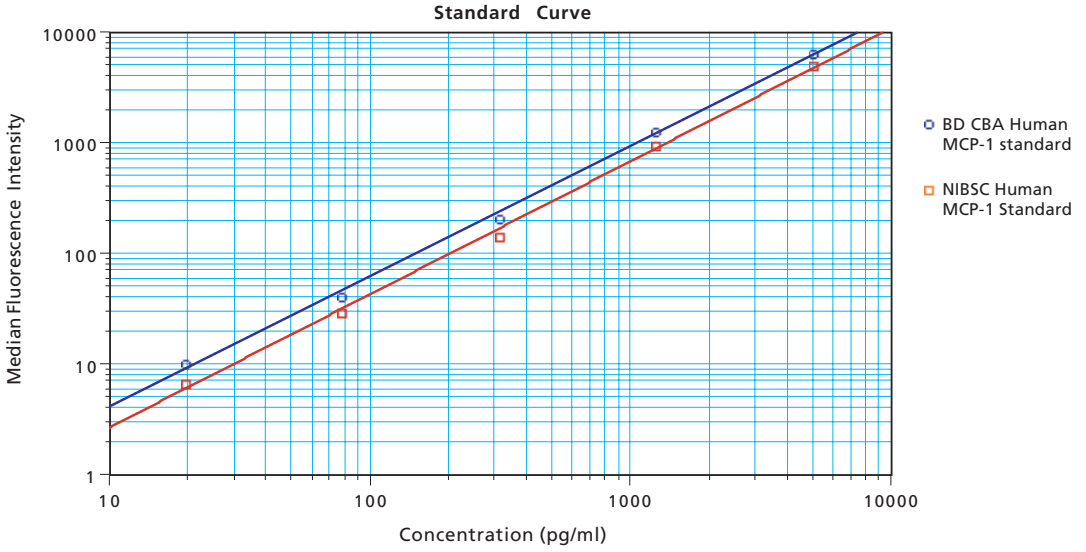

Figure 4. *Titration curve comparing the BD CBA Human CCL2/MCP-1 recombinant standard to the NIBSC/WHO International standard.*

Standardization of BD™ CBA 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 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 researchers.

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 protein measurements obtained with BD CBA kits, BD Biosciences evaluates the assay performance of the standards provided in various BD CBA kits with gold standards from the National Institute for Biological Standards and Control (NIBSC).

The NIBSC Human 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 (ie, 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.

Summarized in the tables below are comparisons between expected concentrations of the International Standards relative to several BD CBA Standards. The resulting data, together with the conversion factors between the BD CBA Standards and the International Standards (ie, nominal mass values) are summarized in **Table 1**. As demonstrated in **Figures 4 and 5**, the performance of both sets of standards in the example shown 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 (ie, 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 protein concentrations present in samples measured by different immunoassays that have been standardized to the same NIBSC/WHO standards.

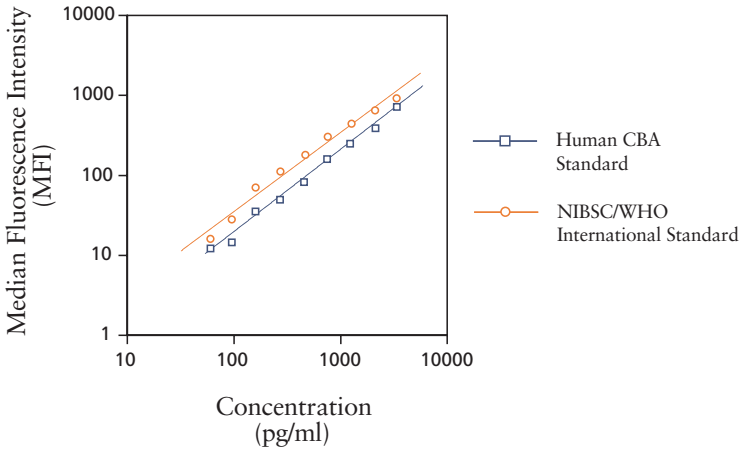

Figure 5. *Titration curve comparing the BD CBA Human IFN-γ recombinant standard to the NIBSC/WHO International standards*

Standardization of BD™ CBA Standards to International NIBSC Standards

Table 1. Conversion factors between the BD CBA Standards and the NIBSC Standards

	HUMAN IL-1β	HUMAN IL-2	HUMAN IL-4	HUMAN IL-5	HUMAN IL-6	HUMAN IL-8	HUMAN IL-10
NIBSC Code Number I.U. Mass units per vial	86/680 100,000 1 µg	86/504 100 7.6 ng	88/656 1,000 100 ng	90/586 5,000 500 ng	89/548 100,000 1 µg	89/520	93/722
Nominal NIBSC concentration (pg/ml)	5,000	5,000	5,000	5,000	5,000	5,000	5,000
Calculated concentration using CBA (pg/ml)	6,420±579	4,108±250	5,155±1,070	4,953±2,334	5,199±842	3,874±1,242	2,617±305
CBA:NIBSC/WHO Mass Conversion Factor	0.78	1.22	0.97	1.01	0.96	1.29	1.91

	HUMAN IL-12P70	HUMAN TNF	HUMAN IFN-γ	HUMAN RANTES	HUMAN MCP-1	HUMAN MIP-1α	HUMAN VEGF	HUMAN bFGF
NIBSC Code Number I.U. Mass units per vial	95/544 10,000 1 µg	87/650 40,000 1 µg	87/586 250 12.5 ng	92/520 100,000 1 µg	92/794	92/518 200 1 µg	01/424 1 µg	90/712 1,600 1 µg
Nominal NIBSC concentration (pg/ml)	5,000	5,000	5,000	5,000	5,000	5,000	5,000	5,000
Calculated concentration using CBA (pg/ml)	3,906±669	5,562±1,633	10,210±550	3,399±476	3,683±390	1,862±215	5,813±941	615±150
CBA:NIBSC/WHO Mass Conversion Factor	1.28	0.90	0.49	1.47	1.36	2.69	0.86	8.12

	MOUSE IL-2	MOUSE IL-4	MOUSE TNF
NIBSC Code Number I.U. Mass units per vial	93/566 10,000 100 ng	91/656 10,000 1 µg	88/532 200,000 1 µg
Nominal NIBSC concentration (pg/ml)	5,000	5,000	5,000
Calculated concentration using CBA (pg/ml)	1,315±184	2,166±195	2,624±334
CBA:NIBSC/WHO Mass Conversion Factor	3.80	2.31	1.91

Non-Human Primate Cross-Reactivity

Several of the antibody pairs used in the BD CBA kits are capable of detecting positive signals for rhesus and cynomolgus macaque samples. The BD CBA results are 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 2. BD CBA Cross-Reactivity with Non-Human Primate Cytokines and Chemokines

DESCRIPTION	RHESUS AND CYNOMOLGUS CROSS-REACTIVITY	CAT. NO.
Human Chemokine Kit I	CXCL8/IL-8, CCL5/RANTES, CCL2/MCP-1	552990
Human Th1/Th2 Cytokine Kit	IL-4, IL-5, TNF, IFN-γ	550749
Human Th1/Th2 Cytokine Kit II	IL-4, IL-6, TNF, IFN-γ	551809
Human Inflammation Kit I	IL-8, IL-6, TNF (IL-1β and IL-12p70 not yet tested)	551811
Non-human Primate Th1/Th2 Kit	IL-2, IL-4, IL-5, IL-6, TNF, IFN-γ	557800

BD FACSArray™ Bioanalyzer is Ideal for BD™ CBA Applications

The BD FACSArray™ bioanalyzer, the latest instrument release from BD Biosciences, provides researchers with a new and compelling platform capable of analyzing cellular and BD™ Cytometric Bead Array assays. Supported by over 1,000 BD Biosciences products, the bioanalyzer is designed for multi-parameter analysis of proteins in immunology and cell biology applications. The system is compact, easy to use, and particularly suited for BD Cytometric Bead Array (CBA) applications.

Sampling speed is delivered by combining a new plate loader technology for sample input and digital electronics for acquisition rates of up to 15,000 events per second. An entire 96-well plate can be turned around in less than 35 minutes, while saving 1000 events per sample well. Each event contains information of up to six parameters. Featuring a dual-laser system, the bioanalyzer allows the use of several extremely bright fluorophores in parallel, thus enabling applications with a wide dynamic range. The BD FACSArray bioanalyzer offers a powerful, yet easy-to-use solution for many avenues of life sciences research. More information can be found online at **www.bdbiosciences.com/bdfacsarray**

Class 1 (I) Laser Product

BD™ CBA Assay Troubleshooting Tips

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

If debris or non-bead events are apparent, switch the threshold to SSC or the dual threshold to FSC and SSC. If the appearance of the debris is not improved, change R1 to an acquisition gate and ensure that the data file save only R1 gated events rather than count R1 events and save all data using existing template configured. CAUTION: An improperly drawn R1 gate can result in loss of data. When detecting debris (FSC/SSC) during sample acquisition, increase the FSC threshold or further wash or dilute samples.

Care in Mixing

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

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.

Recombinant Standards

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

Instrument setup flow cytometer 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 flow cytometers. 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 BD FACSCalibur instruments as well as other instrument setup templates can be found on CBA page at **www.bdbiosciences.com/pharmingen/CBA/**

What is the absolute lowest value that can be reliably detected in plasma or serum?

It is not recommended that researchers report values extrapolated below the lowest standard curve point. This can add mathematical variation to results (due to extrapolation) and cause increased CV's. The optimal condition is for standard curves to be run with additional dilution points (ie, 10, 5, 2.5, 1.25 pg/ml) with the understanding that some proteins will have no signal above background (0 pg/ml) and should be excluded from the final sample analysis. It is not uncommon for cytokines to be present in serum and plasma at levels below the limit of detection for conventional immunoassays. This is due to the fact that most cytokines act locally in the environment around specific sites of immune activity, are highly potent, have short half-lives (in many cases), and act on a broad number of cell types. Thus, it is reasonable to assume that only in abnormal cases would they be measured in high amounts in systemic fluids like serum and plasma. This is not necessarily the case for broader acting proteins like some of the chemokines, anaphylatoxins, hormones, growth factors, etc., which may be produced in higher levels or stabilized in system fluids (longer half-lives) and are more readily measured.

BD™ Cytometric Bead Array Products

DESCRIPTION	CONTENTS	SIZE	CAT. NO.
Human			
Human Allergy/Asthma Mediator	IL-3, IL-4 IL-7, IL-10, GM-CSF	50 tests	Summer 2004
Human Allergy/Asthma Mediator Kit - II	IL-3, IL-4, GM-CSF, G-CSF, Eotaxin	50 tests	Summer 2004
Human Anaphylatoxin Kit	C4a, C3a, C5a	50 tests	552363
Human Angiogenesis Kit	IL-8, bFGF, VEGF, Angiogenin, TNF	50 tests	558014
Human Apoptosis Kit	Cleaved PARP, Bcl-2, Active Caspase-3	50 tests	557816
Human B Cell Activation Kit (Qualitative)	CD79b(Igβ), BLNK, Btk, Syk, PLCγ	50 tests	Summer 2004
Human Chemokine Kit – I	IL-8, RANTES, MIG, MCP-1, IP-10	50 tests	552990
Human Chemokine Kit – II	IL-8, RANTES, MIP-1α, MIP1β, MCP-1	50 tests	558015
Human Inflammation Kit	IL-8, IL-1β, IL-6, IL-10, TNF, IL-12p70	50 tests	551811
Human Th1/Th2 Cytokine Kit	IL-2, IL-4, IL-5, IL-10, TNF, IFN-γ	50 tests	550749
Human Th1/Th2 Cytokine Kit – II	IL-2, IL-4, IL-6, IL-10, TNF, IFN-γ	50 tests	551809
Human T Cell Activation Kit (Qualitative)	TCRz, SLP-76, ZAP70, Pyk2, Itk, PLCγ	50 tests	Summer 2004
Human Quantitative Ig Isotyping	IgG1, IgG2, IgG3, IgG4, IgM, IgA, IgE	50 tests	Summer 2004
Mouse			
Mouse Allergy Kit	IL-3, IL-4, IL-5, IL-9, IL-13, GM-CSF	50 tests	Fall 2004
Mouse Chemokine Kit	RANTES, MIG, KC, MCP-1, MIP-1α, MIP-1β	50 tests	Fall 2004
Mouse Inflammation Kit	IL-6, IL-10, MCP-1, IFN-γ, TNF, IL-12p70	50 tests	552364
Mouse Immunoglobulin Isotyping Kit	Heavy and light chain isotypes of mouse IgG1, IgG2a , IgG2b ,IgG3 , IgA, IgM, IgE	100 tests	550026
Mouse Th1/Th2 Cytokine Kit	IL-2, IL-4, IL-5, TNF, IFN-γ	50 tests	551287
Non-human Primate			
Non-Human Primate Th1/Th2 Cytokine	IL-2, IL-4, IL-5, IL-6, TNF, IFN-γ	50 tests	557800
Rat			
Rat Th1/Th2 Cytokine	IL-2, IL-4, IL-6, IL-10,TNF, IFN-γ	50 tests	Fall 2004
Other			
Human Inflammation Cytokine Standard	IL-8, IL-1β, IL-6, IL-10, TNF, IL-12p70, lyophilized	1 vial	552932
Human Th1/Th2 Cytokine Standard	IL-2, IL-4, IL-5, IL-6, IL-10, TNF, IFN-g, lyophilized	1 vial	551810
Mouse Inflammation Cytokine Standard	IL-6, IL-10, MCP-1, IFN-γ, TNF, IL-12p70	1 vial	620280
Mouse Th1/Th2 Cytokine Standard	IL-2, IL-4, IL-5, TNF, IFN-γ, lyophilized	1 vial	552967
Phosphorylated Stat 1 Kit	Human or Mouse Phosphorylated Stat 1	100 tests	557740
BD CBA Software (v1.4)	Mac and PC Compatible CD-Rom and User's guide	1 CD	550065

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