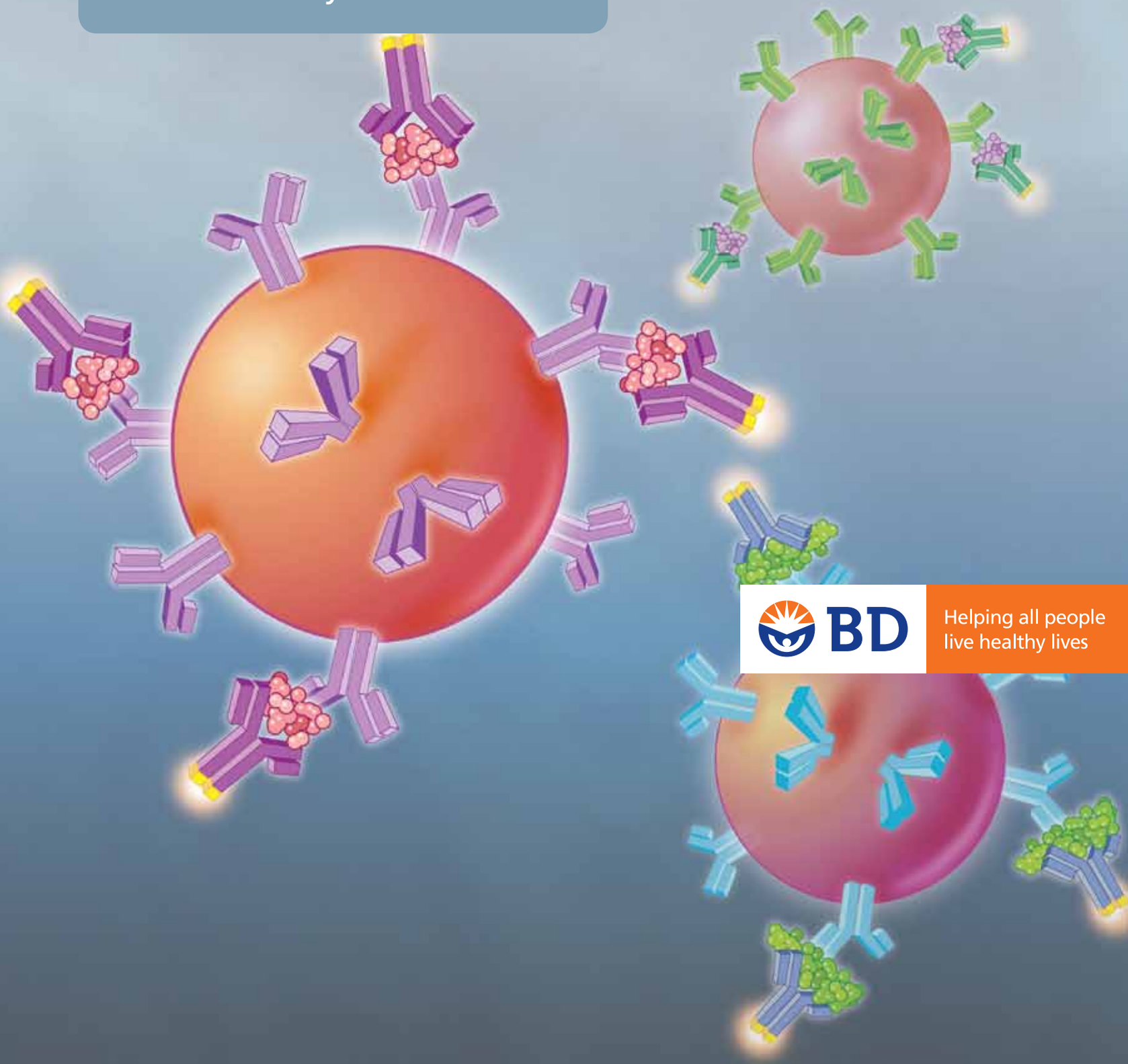
BD Cytometric Bead Array

Multiplexed Bead-Based Immunoassays



BD

Helping all people live healthy lives

Cover Illustration: Fairman Studios, LLC.

BD Cytometric Bead Array: Multiplexed Bead-Based Immunoassays

BD™ Cytometric Bead Array (CBA) is a flow cytometry application that allows users to quantify multiple proteins simultaneously. The BD CBA system uses the broad dynamic range of fluorescence detection offered by flow cytometry and antibody-coated beads to efficiently capture analytes. Each bead in the array has a unique fluorescence intensity so that beads can be mixed and run simultaneously in a single tube. This method significantly reduces sample requirements and time to results in comparison with traditional ELISA and Western blot techniques.

BD CBA solutions are designed for multiplexed analysis, to provide more data using a single sample. Multiplexing is especially useful when only a small amount of sample is available, maximizing the number of proteins that can be analyzed. With BD CBA, up to 30 proteins can be analyzed using just 25 to 50 µL of sample. Other methods such as ELISA and Western blot require a similar amount of sample, but only one protein can be analyzed from the same volume. With the BD CBA Enhanced Sensitivity Flex Set system, it is possible to detect as low as 0.274 pg/mL in a multiplexed assay.

The BD CBA portfolio includes assays for measurement of a variety of soluble and intracellular proteins, including cytokines, chemokines, growth factors, and phosphorylated cell signaling proteins. BD CBA solutions are available in two formats to meet diverse needs:

BD CBA Flex Sets provide an open and configurable method of detection, so that researchers can build their own multiplexes.

BD CBA Kits are preconfigured for achieving consistent results for routine panels.

Available for most BD flow cytometers, BD CBA solutions combine our leadership in instrumentation with innovation in application development to deliver a flexible and robust assay system to fulfill diverse research requirements.

BD CBA Flex Sets—Open and Configurable

The BD CBA Flex Set system provides an open and configurable menu of bead-based reagents designed to make it easy to create multiplex assays.

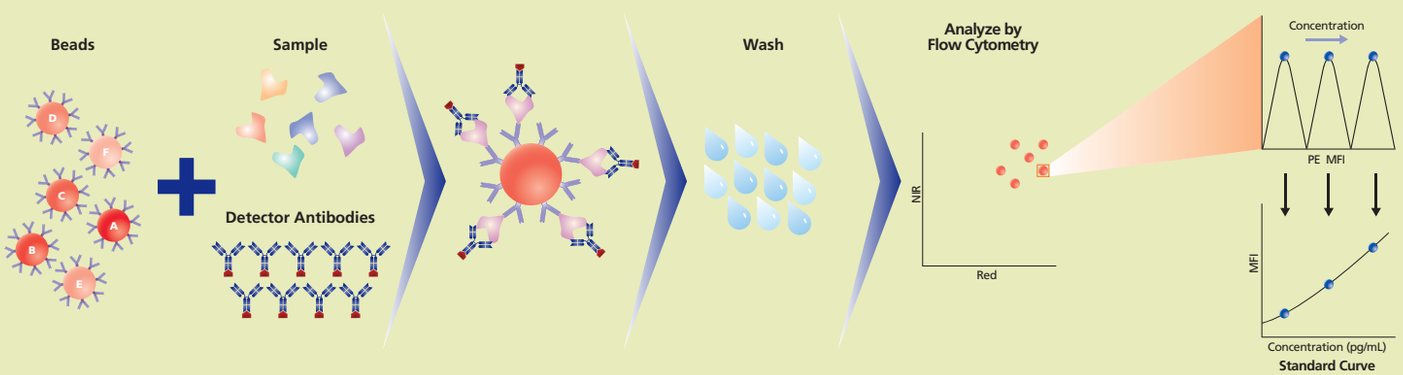
Available specificities include soluble protein assays for detection of human, mouse, or rat cytokines, chemokines, and growth factors; human immunoglobulins; and cell signaling assays for detection of phosphorylated cell signaling proteins.

Up to 30 analytes can be measured simultaneously using the BD CBA Flex Set system on a flow cytometer equipped with 488-nm or 532-nm and 633-nm lasers.

Soluble protein assays

BD CBA Soluble Protein Flex Set assays are available for the detection of cytokines, chemokines, and growth factors from serum, plasma, or tissue culture supernatant samples. These include T-cell differentiation factors, modulators of inflammation, and other key markers for monitoring the immune response.

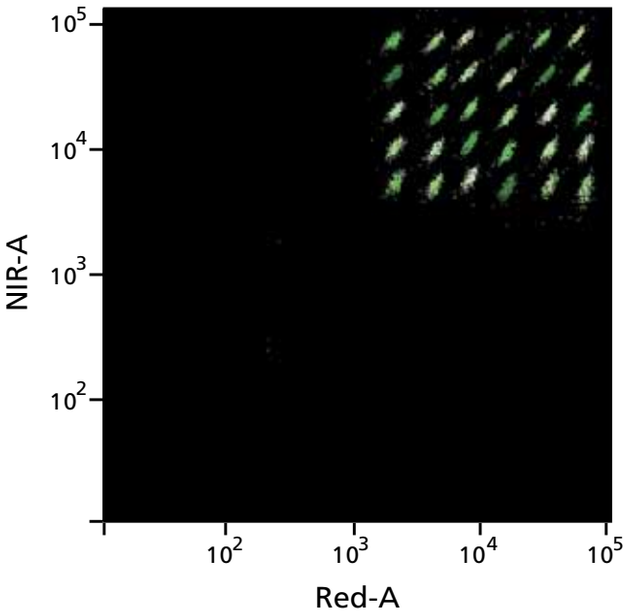
The assays have been formulated to be mixed into any size plex, and they are all sold individually, to provide a highly flexible system. All products are available off-the-shelf so custom orders are not required. Each product area (ie, Human Soluble Protein, Mouse/Rat Soluble Protein, and Human Immunoglobulin) has a unique Master Buffer Kit. All assays within each product area have been verified for performance in single-plex and in multiplexed scenarios to ensure consistent data.



Enhanced sensitivity assays
The BD CBA Enhanced Sensitivity Flex Set assays provide a new level of detection compared to the traditional BD CBA Flex Set assays. The Enhanced Sensitivity Flex Sets can detect as low as 0.274 pg/mL in a multiplexed assay. This breakthrough allows researchers to get quantitative results in samples that were previously below the level of detection. The system includes assays for cytokines involved in inflammation and leucocyte differentiation, which can be combined to create a multiplexed panel. The system provides a reliable, flexible method for quantitative detection of multiple proteins in a single human or mouse sample.

BD CBA Cell Signaling Flex Set Assays
The benefits of BD CBA assays are extended to researchers investigating cell signaling pathways. The assays cover key signaling molecules involved in B-cell and T-cell receptor signaling, as well as other pathways in the immune response such as signaling via growth factor receptors and MAP kinase signaling.

BD CBA 30-plex assay



The assays include a recombinant protein standard that provides an internal control as well as a means to generate a standard curve and subsequent quantitative analysis. The intuitive analysis software generates a numerical readout in relative units/mL for each protein assayed, delivering the answers needed without additional steps. Low inter- and intra-assay CVs allow researchers to have greater confidence in results.

BD CBA products: designed for easy and efficient multiplexing
- Require no assay formulation regardless of plex size
- Deliver quantitative results from a single small volume sample
- Require less total time and less hands-on time compared with competitor bead-based immunoassays
- Offer automated sample acquisition and increased throughput with the plate-based BD FACSArray™ bioanalyzer, BD FACS™ Universal Loader, BD™ High Throughput Sampler, or BD CSampler™ accessory

BD CBA Flex Sets
- Open and configurable bead-based reagents
- Measure up to 30 analytes simultaneously on a flow cytometer with 488-nm or 532-nm and 633-nm lasers

Available assays include:
- Soluble protein assays for detection of human, mouse, or rat cytokines, chemokines, and growth factors, human immunoglobulins
- Enhanced sensitivity assays for cytokine detection <1.0 pg/mL
- Cell signaling assays for detection of phosphorylated cell signaling proteins

Build a Multiplex Step-by-Step

BD CBA Flex Sets make it easy to build a multiplex by following five simple steps. The finished assay can be acquired on a variety of dual-laser flow cytometers and analyzed using FCAP Array™ software.

Instrument	Reporter Parameter	Clustering Parameters
BD FACSVerse™ flow cytometer	PE	CBA Red and CBA NIR
BD Accuri™ C6 flow cytometer*	FL2	FL4 (675/25) and FL3 (780/60)
BD FACSArray bioanalyzer	Yellow	Red and NIR
BD FACSCanto™ II flow cytometer	PE	APC and APC-Cy™7
BD™ LSR II flow cytometer	PE	APC and APC-Cy7
BD FACSAria™ III cell sorter	PE	APC and APC-Cy7
BD FACSCalibur™ flow cytometer	FL2	FL4 and FL3

FCAP Array Parameters for BD CBA Flex Sets

**Requires the BD Accuri Selectable Laser Module (Cat. No. 653126)*

STEP 1: Choose from our menu of Human, Mouse, Rat, Enhanced Sensitivity, and Cell Signaling BD CBA Flex Set Assays.

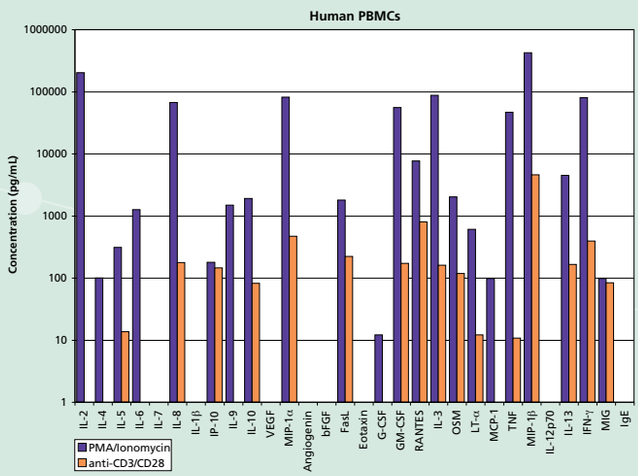
Each BD CBA Flex Set comes with capture beads, detection reagent, and standards. Sufficient reagents are provided to run 100 tests including two standard curves. All assays are available off-the-shelf and ready for mixing. With BD CBA Flex Sets you can get right to work—no custom orders required.

STEP 2: Choose a 100 or 500 test size BD CBA Flex Set Master Buffer Kit.

Each BD CBA Flex Set Master Buffer Kit contains all the assay reagents and instrument setup beads necessary for any size multiplex configured from compatible BD CBA Flex Sets. This means that for running a single-plex assay, a 10-plex assay, or larger, the buffer reagents are optimized to perform with the customized mixture selected and yield the correct number of assay tests.

Protein levels using a BD CBA Flex Set assay

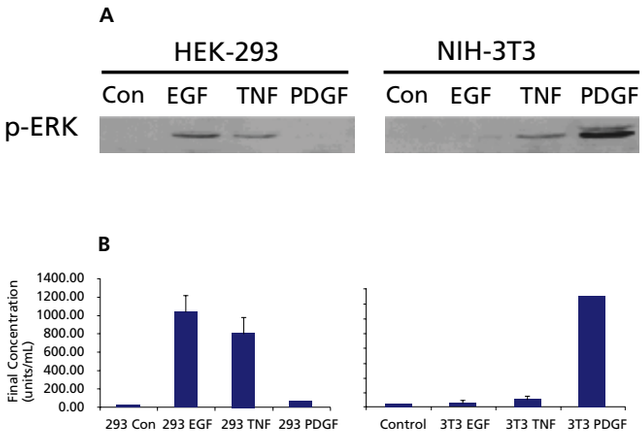
Human peripheral blood mononuclear cells (PBMCs) were stimulated under two different conditions. Supernatants were collected and measured in a BD CBA Flex Set assay (30 plex).



STEP 3: Perform the assay following the instructions in the Master Buffer Kit manual.

STEP 4: Acquire samples on a dual-laser flow cytometer.
BD CBA Flex Set reagents have been verified for performance on a number of BD dual-laser flow cytometry platforms.

STEP 5: Analyze data files using FCAP Array multiplex analysis software.
Use the intuitive FCAP Array software to plot standard curves and calculate sample concentrations.



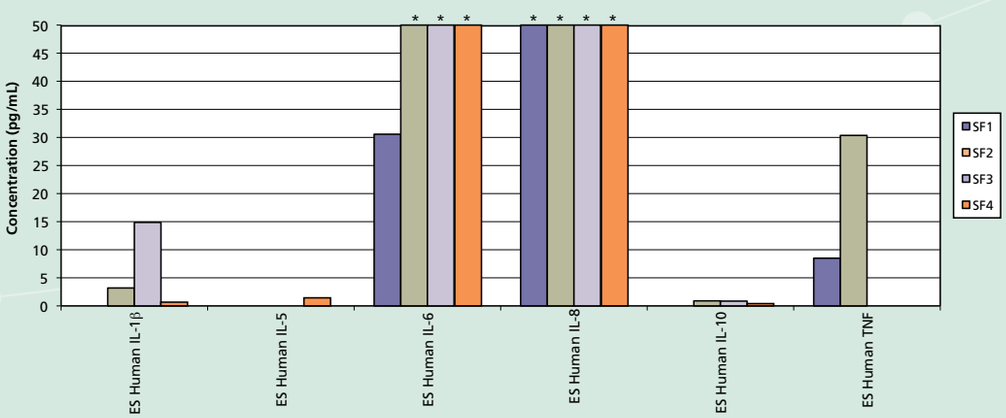
Analysis of phospho-ERK1/2 protein levels in HEK 293 and NIH 3T3 cells in response to EGF, TNF, and PDGF stimulation.

A shows the results of Western blot analysis.

B shows the results of BD CBA Flex Set analysis.

Data courtesy of Dr. Tony Pawson and Dr. Jay Park, Mount Sinai Hospital,Toronto, Canada.

Protein levels using a BD CBA Enhanced Sensitivity Flex Set assay
Synovial fluid samples from autoimmune donors were diluted 1:10 and screened in the Human Enhanced Sensitivity Flex Set system (11-plex). Analytes with detectable amounts are shown in the graph. Most samples (*) had IL-6 or IL-8 levels that were greater than the top point on the standard curve (>200 pg/mL).



BD CBA Kits: Consistent Results with Routine Panels

BD CBA Kits provide preconfigured panels for ultimate ease of use. These kits enable multiplex analysis of complex biological samples on a flow cytometer. In contrast to BD CBA Flex Sets, the kits are preconfigured by functional areas of biology (eg, Th1/Th2 or inflammatory cytokines) to measure up to seven analytes simultaneously using capture beads that contain unique amounts of a single red dye. The unique spectral properties of this dye enable analysis of samples on flow cytometers that have a single 488-nm laser or on dual-laser (488-nm or 532-nm and 633-nm) flow cytometers. Each kit comes complete with all of the buffers and reagents necessary to analyze 80 samples.

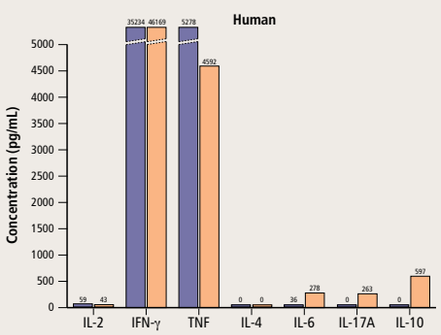
Instrument	Reporter Parameter	Clustering Parameters
BD FACSVerse flow cytometer	PE	CBA Red
BD Accuri C6 flow cytometer	FL2	FL4 (675/25)
BD FACSArray bioanalyzer	Yellow	Red
BD FACSCanto II flow cytometer	PE	APC
BD LSR II flow cytometer	PE	APC
BD FACSAria III cell sorter	PE	APC
BD FACSCalibur flow cytometer (single laser)	FL2	FL3
BD FACSCalibur flow cytometer (dual laser)	FL2	FL4

FCAP Array parameters for BD CBA Kits

Consistent packages, pre-optimized, and ready to use
Each assay has been stringently developed for ease of use, rapid data analysis, sensitivity, reproducibility, and quality. Each antibody pair used in the kits is evaluated for dynamic range, sensitivity, and parallel titration curves to native biological samples. In addition, the assay diluent and wash buffers in each kit have been formulated to reduce detrimental effects of serum and plasma proteins on assay performance.

BD CBA Kits
- Preconfigured kits for consistent results with routine panels
- Available for functional areas of biology such as Th1, Th2, Th17, and inflammatory cytokines
- Measure up to seven analytes simultaneously
- Compatible with flow cytometers that have a 488-nm laser

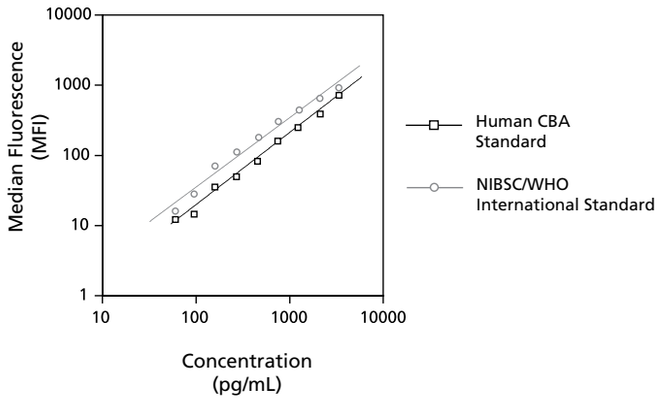
Measurement of Th17 cultures using the BD CBA Th1/Th2/Th17 Kits
$CD4^+$ human memory T cells isolated from whole blood were stimulated with plate-bound anti-CD3 and soluble anti-CD28 alone (blue) or in the presence of recombinant IL-23 (orange).



Comparison of BD CBA Standards to the NIBSC/WHO International Standards

The NIBSC 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). Therefore they cannot be used to establish absolute concentrations for a cytokine preparation. However, these standards do provide a means to facilitate comparisons of cytokine concentration values determined by experiments conducted within different laboratories or methods.

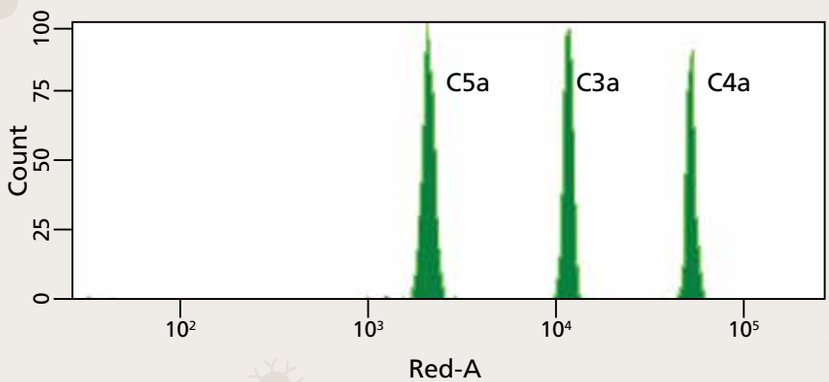
The conversion factor is intended to be a guideline indicating whether a BD CBA assay over- or underestimates analyte concentrations relative to the NIBSC/WHO standards. Researchers are advised to incorporate both sets of standards in their assays if they wish to derive data from the NIBSC/WHO standards. For a table of NIBSC conversion factors, please see the BD CBA Product List included with this brochure.



Titration curve comparing the BD CBA Human IFN-γ recombinant standard to the NIBSC/WHO International Standard.

BD CBA Human Anaphylatoxin Kit

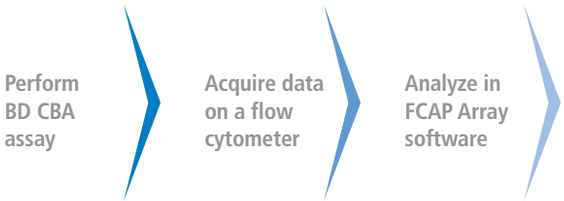
The BD CBA Human Anaphylatoxin Kit (Cat. No. 561418) can be used to quantitatively measure anaphylatoxin C3a, C4a, and C5a (bioactive cleavage products released from C3, C4, and C5 during complement activation) protein levels in a single EDTA plasma or serum sample. The three bead populations are mixed together to form the bead array, which is resolved in a red channel of a flow cytometer.



Simplify Analysis with FCAP Array v3.0 Software

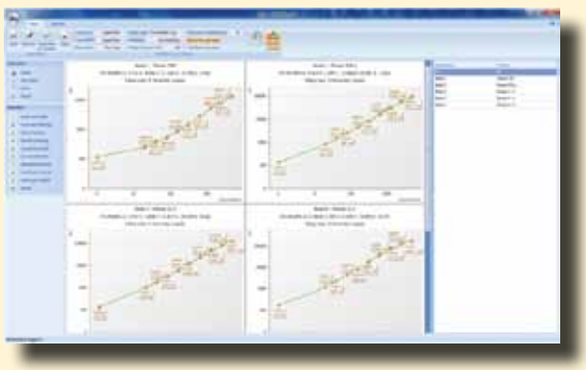
With FCAP Array software (v3.0), sample results are obtained just minutes after performing a CBA experiment. After following the assay protocol, simply collect your data on a flow cytometer, export FCS data files, and then analyze the data using FCAP Array software.

The intuitive linear workflow allows for analysis of a BD CBA assay on any PC workstation. Simply create a new experiment or quickly recall plexes saved from previous experiments. FCAP Array software enables complete analysis of data, interpolation of sample concentrations by comparison to a standard curve, and viewing of results in graphical or tabular format or in a formatted report. Raw data can also be exported for downstream analysis in statistical software.



FCAP Array v3.0 software

- Intuitive layout of samples and assignment to data files
- Standard dilution calculator for fast data entry
- Debris filtering and manual clustering options
- Results viewing by sample or analyte
- Graphical results display
- Compatibility with data files from BD FACS brand flow cytometers and the BD Accuri C6 personal flow cytometer
- Automated workflow for the BD FACSVerse flow cytometer

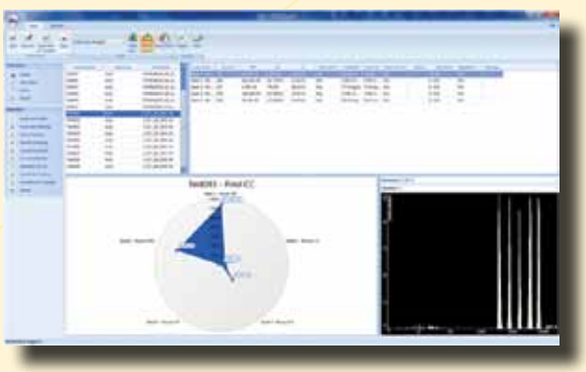


ANALYSIS

Users of the BD FACSVerse flow cytometer equipped with BD FACSuite™ software will enjoy many automated features for even faster results. Based on keywords that are entered prior to acquisition and read by FCAP Array software, analysis begins as soon as the FCS data files are identified.

These keywords include identification of sample type (eg, standard, test sample, or control), identification of a plex name that automatically assigns a plex if a match is found in the FCAP Array plex library, and the sample dilution factor. This automated workflow also reads the Sample ID and Experiment Name directly from the FCS data file, providing consistent identification of a sample throughout the entire workflow and verification that all data files are from a related experiment.

FCAP Array v3.0 software can use data files exported in FCS 2.0 or 3.0 formats, providing analysis capability for major BD instrument platforms that use BD FACSuite, BD FACSDiva™, BD FACSArray™, BD Accuri™ C6, or BD CellQuest™ Pro software. For FCS 3.0 data files, FCAP Array software is capable of properly displaying compensated data on a log scale. In the event that the software cannot identify gates for the bead clusters, intuitive gating tools are available within FCAP Array software to filter out debris or to create manual gates. This provides significant time savings and ensures data integrity.



Additional Options to Enhance Flexibility

BD CBA Functional Beads to conjugate for unique requirements

BD CBA Flex Set Functional Beads are unconjugated beads that allow researchers to conjugate their own antibody or protein of interest using sulfo-SMCC chemistry. The conjugation procedure takes less than four hours using common laboratory supplies along with the buffers in the BD CBA Functional Bead Conjugation Buffer Kit.

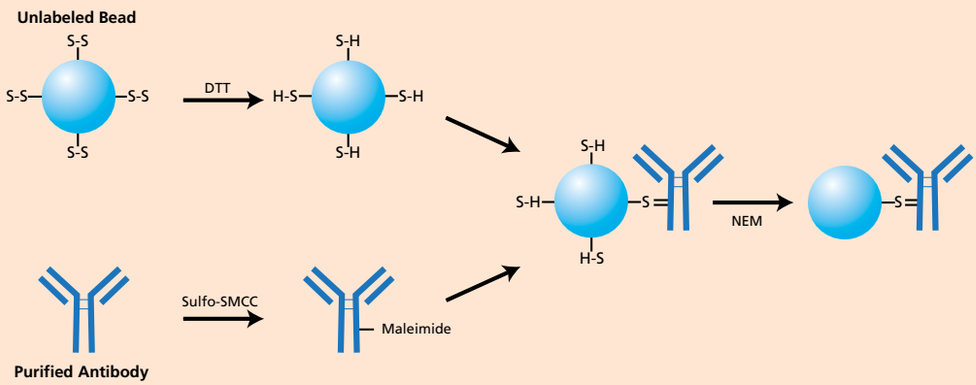
This is an ideal platform for converting existing ELISAs into bead-based immunoassays that can be mixed with our portfolio of BD CBA Flex Set assays. The availability of multiple bead positions enables creation of novel multiplex panels, while the ability to prepare up to 1,000 tests in a single reaction ensures consistency across a large number of tests.

The procedure to conjugate a protein to a functional bead consists of four major steps:

- Step 1: Bead Preparation
- Step 2: Protein Modification
- Step 3: Buffer Exchange to Remove Unreacted Components
- Step 4: Protein Conjugation

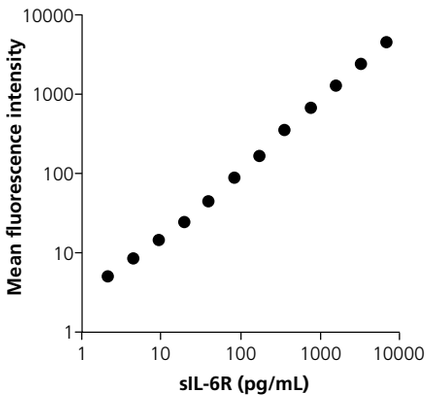
The estimated time to completion for the entire conjugation procedure is 3.5 hours.

Overview of the functional bead conjugation procedure



Advantages

- Simple conjugation procedure completed in less than 4 hours.
- Conjugation reaction requires less than 100 µg of protein at a concentration of 1 mg/mL.
- Multiple bead populations that can be used individually or multiplexed as needed.
- Ability to conjugate any protein molecule containing a free amino group to the beads.
- Compatible with a wide selection of flow cytometers for ease of analysis.
- Specific reagents available for confirming the success of conjugation reactions.
- Supporting reagents and procedures available for performing instrument setups and assays.

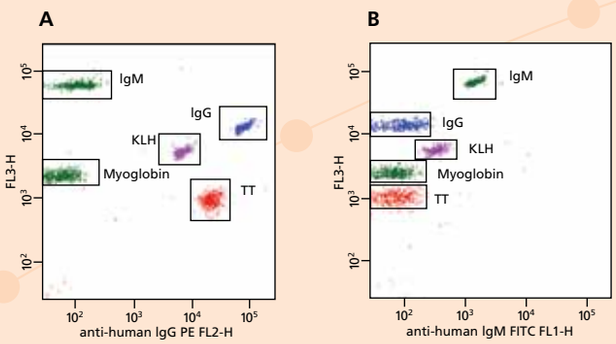


Standard curve for a soluble IL-6 receptor assay generated using BD CBA Functional Bead E4 following the conjugation procedure in the BD CBA Functional Bead Conjugation Buffer Set manual.

Data courtesy of Joseph Cannon and Gloria Sloan, Medical College of Georgia.

A novel assay from the Custom Technology Team

In the assay shown, spectrally distinct beads coated with keyhole limpet hemocyanin (KLH) or tetanus toxoid (TT) were incubated with a test serum to detect the presence of antibodies to the two antigens. Incubating each sample with a mix of anti-IgG or anti-IgM detection antibodies that are labeled with unique fluorochromes allows for determination of the isotype of anti-KLH or anti-TT antibodies. Additional beads coated with IgG or IgM serve as positive controls, and a bead coated with myoglobin serves as the negative control. The data shown demonstrates that the test serum is positive for TT and KLH specific IgG antibodies (A), but positive only for KLH specific IgM antibodies (B). All measurements are made from one tube, saving time and sample.



Services and Support

BD Biosciences instruments and reagents are backed by a world-class service and support organization with unmatched flow cytometry experience. Our integrated approach combines high-content bioimaging and flow cytometry instrumentation with trusted, certified reagents, and advanced applications. The BD Biosciences tools enable our customers to discover more and obtain the most complete picture of cell function, and at the same time experience improved workflow, ease of use, and optimal performance.

Researchers come to BD Biosciences not only for quality products, but as a trusted lab partner. Our repository of in-depth, up-to-date knowledge and experience is available to customers through comprehensive training, application and technical support, and expert field service.

The Custom Technology Team
Mobilizing technology for research applications requires close collaboration. The Custom Technology Team (CTT) at BD Biosciences works with customers to provide solutions through custom reagents, panels, or assay protocols. Staffed by leading scientists with a breadth and depth of scientific and technical expertise, the CTT will coordinate with researchers to study the problem at hand, make recommendations, and help implement the solutions. In this way, BD Biosciences technical know-how is translated into practical solutions that allow customers to focus on research.

Technical application support
BD Biosciences technical applications support specialists are available to provide field- or phone-based assistance and advice. Expert in a diverse array of topics, BD technical application specialists are well equipped to address customer needs in both instrument and application support.

Choose the BD CBA product for your needs

BD CBA Flex Sets
- Open and configurable bead-based reagents
- Measure up to 30 analytes simultaneously on a flow cytometer with 488-nm or 532-nm and 633-nm lasers

Available assays include:
- Soluble protein assays for detection of human, mouse, or rat cytokines, chemokines and growth factors, human immunoglobulins
- Enhanced sesitivity assays for cytokine detection <1.0 pg/mL
- Cell signaling assays for detection of phosphorylated cell signaling proteins

BD CBA Kits
- Preconfigured kits for consistent results with routine panels
- Available for functional areas of biology such as Th1, Th2, Th17, and inflammatory cytokines
- Measure up to seven analytes simultaneously
- Compatible with flow cytometers that have a 488-nm laser

BD Functional Beads
- Unconjugated beads that allow researchers to conjugate their own antibody or protein of interest
- Enable the creation of novel bead assays

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