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BD Cytometric Bead Array (CBA) Frequently Asked Questions

What kinds of BD™ CBA products are available?

BD CBA products are available as:
- Kits
- Flex Sets
- Enhanced Sensitivity Flex Sets
- Functional Beads

1. **BD CBA kits** contain all reagents necessary for detecting the indicated specificities.

   The following kits are available (80 tests per kit, unless indicated otherwise):

Kit Name	Cat. No.	Specificities
Human Chemokine	552990	IL-8, RANTES, MIG, MCP-1, IP-10
Human Inflammatory Cytokine	551811	IL-8, IL-1β, IL-6, IL-10, TNF, IL-12p70
Human Th1/Th2 Cytokine	550749	IL-2, IL-4, IL-5, IL-10, TNF, IFN-γ
Human Th1/Th2 Cytokine (II)	551809	IL-2, IL-4, IL-6, IL-10, TNF, IFN-γ
Human Th1/Th2/Th17	560484	IL-2, IL-4, IL-6, IL-10, IL-17A, IFN-γ, TNF
Mouse Inflammation	552364	IL-6, IL-10, MCP-1, IFN-γ, TNF, IL-12p70
Mouse Th1/Th2 Cytokine	551287	IL-2, IL-4, IL-5, IFN-γ, TNF
Mouse Th1/Th2/Th17	560485	IL-2, IL-4, IL-6, IL-10, IL-17A, IFN-γ, TNF
Mouse Immunoglobulin Isotyping	550026	IgG1, IgG2a, IgG2b, IgG3, IgA, IgM, IgE, kappa and lambda light chains, 100 tests
Non-human Primate Th1/Th2 Cytokine	557800	IL-2, IL-4, IL-5, IL-6, TNF, IFN-γ

2. **BD CBA Flex Sets** each detect a distinct protein specificity and can be mixed together to form a multiplex experiment (up to 30 specificities). To view a list of Flex Set specificities and multiplex compatibility, visit http://www.bdbiosciences.com/reagents/cytometricbeadarray/products/flexsets.jsp

   Each Flex Set contains capture beads, PE-conjugated detector antibody, and a recombinant standard. The following buffers (called Master Buffer Kits) are recommended when using BD CBA Flex Sets. The Flex Sets are organized by product families and are optimized for a given Master Buffer Kit. The Master Buffer Kits also include beads for setting up the flow cytometer.

Flex Set Type	Buffer Kit Cat. No.	Number of Tests
Human soluble protein (except Human Ig Flex Sets)	558264 558265	100 500
Human Ig	558683	100
Mouse/rat soluble protein	558266 558267	100 500
Cell signaling	560005 560006	100 500

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3. **BD CBA Enhanced Sensitivity Flex Sets** are similar to the original format, except with greater sensitivity than the equivalent Flex Set reagents. Each Enhanced Sensitivity Flex Set includes capture beads, part A of the detector reagent, and a recombinant standard. Part B of the detector reagent is supplied in the corresponding Master Buffer Kit.

   The Master Buffer Kits recommended for the Enhanced Sensitivity Flex Sets are Cat. No. 561521 (100 tests) or Cat. No. 561523 (500 tests).

4. **BD CBA Functional Beads** are for investigators wishing to couple an antibody, protein, or peptide to the beads to create a Flex Set with a specificity of their choice. A separate buffer set is recommended for coupling reagents to the Functional Beads (Functional Bead Conjugation Buffer Set, Cat. No. 558556, 15 reactions).

   Thirty distinct bead positions are available for the BD CBA Functional Beads. To view the complete list, visit http://www.bdbiosciences.com/reagents/cytometricbeadarray/products/flexsets.jsp

   Note: BD CBA Flex Sets, Enhanced Sensitivity Flex Sets, and Functional Beads are designed to be used as integral units. Do not mix components from different batches or kits.

Supplemental reagents:

BD CBA Assay Diluent (Cat. No. 560104) for the Human and Mouse/Rat Soluble Protein Master Buffer Kits. Not recommended for the Cell Signaling Master Buffer Kit or BD CBA kits.

Wash Buffer (Cat. No. 560105) for all BD CBA Flex Sets and BD CBA kits.

A 30-plex bead mixture (Cat. No. 558522, 10 tests) can be used as a control to verify the assay setup procedure for BD CBA Flex Sets or Functional Beads.

Recombinant standards for many of the BD CBA kits, Flex Sets, and Enhanced Sensitivity Flex Sets are also available separately.

The enhanced sensitivity detection reagent (part B) is available separately (Cat. No. 561519) for the Enhanced Sensitivity Flex Sets when existing reconstituted reagent (from the Enhanced Sensitivity Master Buffer Kit) has been used or has extended beyond the recommended storage time.

Which BD instrument platforms are compatible with BD CBA assays?

Product Type	Platform
BD CBA kits	BD FACScan™, BD FACSCalibur™, BD FACSArray™ BD FACSCanto™, BD FACSCanto™ II, BD™ LSR II, BD LSRFortessa™, BD FACSAria™, BD FACSAria™ II, BD FACSAria™ III
Flex Sets	BD dual-laser FACSCalibur, BD FACSArray, BD FACSCanto, BD FACSCanto II, BD LSR II, BD LSRFortessa, BD FACSAria™, BD FACSAria™ II, BD FACSAria™ III

For the BD™ LSR or other digital flow cytometers, email ResearchApplications@bd.com for details. BD CBA assays are not recommended for stream-in-air cell sorters such as the BD FACStar™, BD FACSVantage™, and BD Influx™ systems.

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How do I set up a BD CBA assay on the instrument in my lab?

Instructions and templates for instrument setup are available for download http://www.bdbiosciences.com/cbasetup

For BD FACSAria and BD FACSAria III instruments, use the BD FACSAria II template and instructions. For the BD FACSCanto instrument, use the BD FACSCanto II template and instructions.

For setting up a BD CBA assay on the BD FACSCalibur instrument, two options are available, single-laser or dual-laser setup. For instructions, go to http://www.bdbiosciences.com/cbasetup.

Note: To help prevent data loss, do not use the instructions for the single-laser setup with templates for dual-laser setup.

How can I analyze data for the BD CBA assays?

All BD CBA assays can be analyzed with FCAP Array™ software (Cat. No. 645447 for PC, Cat. No. 641488 for Mac®).

BD CBA software (Cat. No. 550065) has been discontinued. For questions, email ResearchApplications@bd.com.

BD CBA assays can be analyzed with conventional flow cytometry analysis software by gating on the appropriate bead clusters and measuring the PE median value for the bound analyte. Both FITC and PE median values are measured for the Mouse Ig isotyping kit.

What are the physical properties of the BD CBA beads?

The BD CBA beads are hard-dyed polystyrene-based microspheres with an average diameter of 7.5 µm. Capture beads for the BD CBA kits contain one dye, whereas the capture beads for the Flex Sets, Enhanced Sensitivity Flex Sets, and Functional Beads contain two dyes, permitting simultaneous detection of up to 30 specificities. The CBA beads are excited by 488 nm or 532 nm and 633 nm or 635 nm lasers, with fluorescence emissions at 576 nm, 660 nm, and >680 nm.

Is the BD Mouse Ig isotyping CBA kit (Cat. No. 550026) quantitative?

No, this is an isotyping kit for qualitative analysis only.

Which blood anticoagulants can be used for preparation of serum and plasma samples?

We have not extensively tested plasma drawn in lithium or heparin for the BD CBA assay, but we do not expect performance to differ greatly from EDTA-treated plasma. There are differences in the protein spike recovery and linearity between serum and plasma due to differences in the sample protein levels and composition. This should not affect the ability to generate a result for any native protein present in the sample, nor affect the comparison of results between the samples tested in the assay. However, if concerned, investigators might test the recovery or linearity of the assay for their sample type during the process validation to assess any sample-induced variation in results.

In general, serum samples should be centrifuged to remove any precipitates before preparing dilutions for incubation with the capture beads.

Will hemolysis hinder results in BD CBA assays?

A small amount of hemolysis is unlikely to be a problem because the samples are washed before acquisition.

Can BD CBA samples be fixed prior to analysis?

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Samples should not be fixed before performing a BD CBA experiment. After the BD CBA assay protocol is complete, samples may be fixed with 1% paraformaldehyde, washed, and acquired on the cytometer. This might affect the overall assay signal and possibly the sensitivity for various proteins, but will not completely destroy the assay signal. Fixation has been shown to be effective in other laboratories (feedback we have been given). This has not been routinely performed at BD, and prior to CBA analysis, we recommend that investigators validate this method before implementing it with valuable samples.

How are spike recoveries of cytokines in serum or plasma determined by BD CBA assays?

Spike recovery for some cytokines is low in serum because serum has many factors that can hinder detection of soluble proteins by immunoassays. For spike-recovery assays at BD, serum or plasma is diluted 1:2 with assay diluent. An equal volume of recombinant protein in assay diluent is added to give three final concentrations of 625 pg/mL, 156 pg/mL, and 40 pg/mL (performed in triplicate).

Generally the more protein spiked, the better the recovery. The 1:4 dilution in assay diluent helps, probably by diluting out inhibitory factors. In some cases this may improve recovery by 40 to 80%. The equilibration time between spiking and performing the assay has not been investigated thoroughly, but is about 30 minutes in a typical workflow. Some cytokines such as IFN-γ can lose 50% in recovery if the spiked serum/plasma sample is kept overnight at 4°C and probably more so in freeze/thaw samples. Even though the spike recovery may be low, linearities are very good upon dilution.

Spiking into individual samples of sera/plasma can be performed, but donor-donor variation is large, eg, 20 to 80% for some cytokines, and difficult to predict. Spike recoveries using donor-pooled (>700 donors) sera/plasma provide more interexperiment consistency.

What is the stability of BD CBA standards after reconstitution?

We recommend using reconstituted standard, CBA soluble proteins, within 12 hours. We do not recommend freezing and thawing reconstituted standards. Cell signaling CBA standards, after reconstitution, stable for 3 months when stored at 4 °C.

What is the source of the recombinant protein standards in the BD CBA assays?

The recombinant proteins are expressed within insect cells or *E. coli*.

What is the serum enhancement buffer (supplied in the human CBA kits) or the capture bead diluent for serum or plasma (supplied in the human soluble protein Master Buffer Kit, for Flex Sets)?

These buffers contain agents that block human anti-mouse antibodies that are endogenous in many people's serum. These antibodies can sometimes lead to false positives by reacting with the capture and/or detector antibodies used in the assay.

The Human Chemokine kit (Cat. No. 552990) does not include the serum enhancement buffer because it reduces the assay signal for some specificities by >50%. While the root cause has not been fully characterized, the serum enhancement buffer affects detection of chemokines more than that of cytokines.

The workflow for the BD CBA Flex Sets differs from that of the BD CBA kits, so the capture bead diluent for serum/plasma doesn’t impact the chemokine Flex Set assays to the same extent.

Can BD CBA cytokine or chemokine assays be used with tissue lysates?

We have not tested BD CBA kits and Flex Sets on tissue samples for the detection of cytokines or chemokines. However, there are papers describing the use of BD CBA products with tissue homogenates. Some recent references include:

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Milano F, Jorritsma T, Rygiel AM, et al. Expression pattern of immune suppressive cytokines and growth factors in oesophageal adenocarcinoma reveal a tumor immune escape-promoting microenvironment. *Scand J Immunol.* 2008;68:616-623.
Bhowmick S, Duseja R, Das S, Appaiahgiri MB, Vrati S, Basu A. Induction of IP-10 (CXCL10) in astrocytes following Japanese encephalitis. *Neurosci Lett.* 2007;414:45-50.
Ghoshal A, Das S, Ghosh S, et al. Proinflammatory mediators released by activated microglia induces neuronal death in Japanese encephalitis. *Glia*. 2007;55:483-496.
Tong J, Bandukwala HS, Clay BS, et al. Fas-positive T cells regulate the resolution of airway inflammation in a murine model of asthma. *J Exp Med.* 2006;203:1173-1184.
Mansour MK, Yauch LE, Rottman JB, Levitz SM. Protective efficacy of antigenic fractions in mouse models of cryptococcosis. *Infect Immun*. 2004;72:1746-1754.

For detection of analytes within tissue homogenates, spike-recovery assays using the supplied standard into the tissue lysate/homogenate can assist in establishing a positive control and/or checking for the presence of inhibitors. Sample dilutions might help to dilute out inhibitory factors, and native lysates might be preferable over denatured samples.

How do I treat viscous samples for a BD CBA cell signaling assay?

Viscous samples can be sheared with either sonication, passing several times through a 26-gauge needle, or digestion with DNase, as described in the Cell Signaling Master Buffer kit instruction manual. For sonication, use a probe sonicator and reasonably large volume of sample. A simple and rapid approach for many samples is to use DNase. High-quality DNase that is protease free is beneficial for preventing digestion of the target protein. Commercial sources include Ambion (Cat. No. 2222) and USB (Cat. No. 78311). Typically you don't need to add very much and it works very quickly.

In general, samples for BD CBA cell signaling assays should be centrifuged to remove any precipitates before taking 50 µL for incubation with the capture beads.

What are recommended as positive control cell lysates for BD CBA cell signaling Flex Sets?

General activators such as pervanadate (for tyrosine sites) and PMA or calyculin (serine, threonine sites) will give large phosphorylation signals. Cell lines also tend to generate larger responses than primary cells.

Specificity	Cell type	Activation
Btk	Ramos	pervanadate or anti-Ig
eNOS	HE	pervanadate
ERK	Ramos, HE Jurkat A431	pervanadate pervanadate or anti-CD3/CD28 EGF
Itk	Jurkat	pervanadate or anti-CD3/CD28
JNK	Jurkat, HE HeLa 3T3	pervanadate anisomysin osmotic shock
P38	Jurkat HE 3T3	pervanadate or anti-CD3/CD28 pervanadate osmotic shock
PLCg	Jurkat HE	pervanadate or anti-CD3/CD28 pervanadate
Rsk	HE A431	pervanadate EGF

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STAT1	Jurkat A431 Daudi	pervanadate EGF IFN
Syk	Ramos	pervanadate or anti-Ig
ZAP70	Jurkat, PBMC	pervanadate or anti-CD3/CD28

Which molecules (eg, antibodies, DNA, etc) can be conjugated to the BD CBA Functional Beads?

Within BD Biosciences, Functional Bead conjugations have been performed with antibodies only.

Are there any references citing BD CBA assays?

Yes, many papers have used BD CBA assays. Some citations from 2009 are shown below. Please email ResearchApplications@bd.com for other references or search for “cytometric bead array” on google.scholar.com.

Li Q, Oshige H, Zhen Y, et al. Interleukin-5 and interleukin-10 are produced in central nervous system tumor cysts. *J Clin Neurosci*. 2009;16:437-440.
Unsinger J, McDonough JS, Schultz LD, Ferguson TA, Hotchkiss RS. Sepsis-induced human lymphocyte apoptosis and cytokine production in “humanized” mice. *J Leukoc Biol*. 2009;86:219-227.
Wilson JE, Katkere B, Drake JR. *Francisella tularensis* induces ubiquitin-dependent major histocompatibility complex class II degradation in activated macrophages. *Infect Immun*. 2009;77:4953-4965.
Rhee KJ, Wu S, Wu X, et al. Induction of persistent colitis by a human commensal, enterotoxigenic *Bacteroides fragilis*, in wild-type C57BL/6 mice. *Infect Immun*. 2009;77:1708-1718.
Chen GY, Tang J, Zheng P, Liu Y. CD24 and Siglec-10 selectivity repress tissue damage-induced immune response. *Science*. 2009;323:1722-1725.

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