BD Cytometric Bead Array (CBA) Human Soluble Protein Master Buffer Kit

Instruction Manual

Catalog No. 558264 (100 Tests)
Catalog No. 558265 (500 Tests)

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About this kit

This section covers the following topics:

Purpose of the kit

Use of the kit

The BD™ CBA Human Soluble Protein Flex Set System employs particles with discrete fluorescence intensities to detect soluble analytes at very low concentrations. The working assay range for most analytes in this system is 10 to 2,500 pg/mL.

The BD™ CBA Human Soluble Protein Master Buffer Kit contains all of the supporting reagents necessary to perform an assay using a BD CBA Human Soluble Protein Flex Set. The buffers and instrument setup reagents provided in this kit have been optimized for analysis of analytes in tissue culture supernatants, plasma, and serum samples. The BD CBA Human Soluble Protein Master Buffer Kit provides sufficient reagents for the quantitative analysis of 100 samples and 10 instrument setup procedures (Catalog No. 558264) or 500 samples and 10 instrument setup procedures (Catalog No. 558265).

Principle of CBA assays

BD CBA assays provide a method of capturing a soluble analyte or set of analytes with beads of known size and fluorescence, making it possible to detect analytes using flow cytometry.

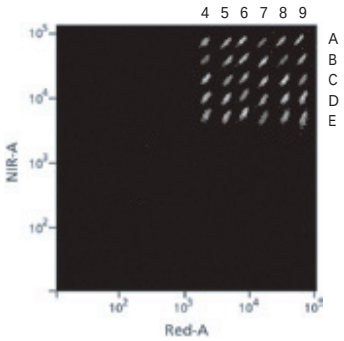
Each capture bead in the BD CBA Human Soluble Protein Flex Set system has a distinct fluorescence and is coated with a capture antibody specific for a soluble protein. The detection reagent is a mixture of phycoerythrin (PE)-conjugated antibodies, which provides a fluorescent signal in proportion to the amount of bound analyte.

When the capture beads and detection reagent are incubated with standards or unknown samples containing recognized analytes, sandwich complexes (capture bead + analyte + detection reagent) are formed.

These complexes can be measured using flow cytometry to identify particles with fluorescence characteristics of both the bead and the detector.

Principle of this assay

A BD CBA Human Soluble Protein Flex Set Capture Bead is a single bead population with distinct fluorescence intensity and is coated with a capture antibody specific for a soluble protein. The bead population is resolved in two fluorescence channels of a flow cytometer. For specific instruments and the channels used to resolve the beads, see the flow cytometers listed in Materials required but not provided (page 15).



Each bead population is given an alphanumeric position designation indicating its position relative to other beads in the BD CBA Human Soluble Protein Flex Set System. Beads with different positions can be combined in assays to create a multiplex assay. The intensity of PE fluorescence of each sandwich complex reveals the concentration of that particular analyte. After acquiring samples on a flow cytometer, use FCAP Array™ software to generate results in graphical and tabular format.

Advantages over ELISA

The broad dynamic range of fluorescence detection via flow cytometry and the efficient capturing of multiple analytes via suspended particles enable the BD CBA Flex Set system to obtain the concentration of an unknown in substantially less time and using fewer sample dilutions compared to conventional ELISA methodology.

- The BD CBA Human Soluble Protein Flex Set assays allow for multiplexed analysis of multiple proteins from a single sample.
- A single set of diluted standards is used to generate a standard curve for each analyte.

Limitations

Assay limitations

The BD CBA Human Soluble Protein Flex Set System is not recommended for use on stream-in-air instruments for which signal intensities may be reduced, adversely affecting assay sensitivity. Stream-in-air instruments include the BD FACStar™ Plus, BD FACSVantage™, and BD Influx™ flow cytometers (BD Biosciences).

Quantitative results or protein levels for the same sample or recombinant protein run in ELISA and BD CBA assays might differ. A spike recovery assay can be performed using an ELISA standard followed by BD CBA analysis to assess possible differences in quantitation.

When several BD CBA Human Soluble Protein Flex Set assays are multiplexed, it is possible that the background (MFI of the 0 pg/mL standard point) might increase and the overall assay signals of other standard points might be reduced. This can result in lower dynamic range or

loss in sensitivity in some assays. This effect might be greater as more assays are added to the multiplex.

For assays that will be acquired on a BD FACSCalibur™ flow cytometer, we recommend that additional dilutions (1:512 and 1:1024) of the standard be prepared, since it is possible that in multiplex experiments containing a large number of assays, the Top Standard, 1:2, and 1:4 standard dilution cannot be analyzed by FCAP Array software. In those cases, the Top Standard, 1:2, and 1:4 standard dilutions can be run on the experiment but might need to be excluded from the final analysis in FCAP Array software.

The Human Soluble Protein Master Buffer has been optimized for use with the BD CBA Human Soluble Protein Flex Sets and should not be used with any non-Human Soluble Protein Flex Sets. For an assay compatibility chart for the BD CBA Human Soluble Protein Flex Sets, please visit bdbiosciences.com/cbasetup.

Kit contents

Contents

The kit contains the following components sufficient for 100 tests (Catalog No. 558264) and 500 tests (Catalog No. 558265).

Reagent	Quantity (558264)	Quantity (558265)
Assay Diluent	1 bottle, 30 mL	1 bottle, 150 mL
Capture Bead Diluent	1 bottle, 5 mL	1 bottle, 30 mL
Detection Reagent Diluent	1 bottle, 5 mL	1 bottle, 30 mL
Capture Bead Diluent for Serum/Plasma	1 bottle, 5 mL	1 bottle, 30 mL
Wash Buffer	1 bottle, 130 mL	1 bottle 650 mL
Instrument Setup Bead A1	1 vial, 0.25 mL	1 vial, 0.25 mL
Instrument Setup Bead A9	1 vial, 0.25 mL	1 vial, 0.25 mL
Instrument Setup Bead F1	1 vial, 1.0 mL	1 vial, 1.0 mL
Instrument Setup Bead F9	1 vial, 0.25 mL	1 vial, 0.25 mL
PE Instrument Setup Bead F1	1 vial, 0.25 mL	1 vial, 0.25 mL
PE Positive Control Detector	1 vial, 0.5 mL	1 vial, 0.5 mL

Storage and handling

Storage

Store all kit components at 2 to 8°C. Do not freeze.

Appearance: The visual appearance of Assay Diluent may range in color from red to yellow/orange. The visual appearance of ELISA Dilution reagent may range in color from clear to cloudy white.

Warning

All components in this kit contain phosphate buffered solution containing protein and sodium azide. Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.

Note: Source of all serum proteins is from USDA-inspected abattoirs located in the United States.

Please refer to http://regdocs.bd.com to access safety data sheets (SDS).

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Before you begin

This section covers the following topics:

Workflow overview

Workflow

The overall workflow consists of the following steps.

Step	Description
1	Preparing Human Flex Set Standards (page 18)
2	Mixing Human Soluble Protein Flex Set Capture Beads (page 20)
3	Diluting test samples (page 23)
4	Preparing Human Soluble Protein Flex Set PE Detection Reagents (page 25) **Note:** Can be prepared during the first incubation in step 6 below.
5	Performing instrument setup with Instrument Setup Beads, if necessary (instructions can be found at bdbiosciences.com/cbasetup) **Note:** Can be performed during one of the incubations in step 6.
6	Performing the Human Soluble Protein Flex Set Assay (page 30)
7	Acquiring samples (instructions can be found at bdbiosciences.com/cbasetup)
8	Data analysis (page 34)

Incubation times

To help you plan your work, the incubation times are listed in the following table.

Procedure	Incubation time
Preparing standards	15 minutes
Preparing Capture Beads (serum and plasma samples only)	15 minutes
Assay Procedure	
• First incubation–Capture Beads	1 hour
• Second incubation–PE Detection Reagent	2 hours

Required materials

Materials required but not provided

In addition to the reagents provided in the BD CBA Human Soluble Protein Master Buffer Kit and the BD CBA Human Soluble Protein Flex Set, the following items are also required.

- A dual-laser flow cytometer equipped with a 488-nm or 532-nm and a 633-nm or 635-nm laser capable of distinguishing 576-nm, 660-nm, and >680-nm fluorescence. The following table lists examples of compatible instrument platforms.

Flow cytometer	Reporter channel	Bead channels
BD FACSArray™	Yellow	Red and NIR
BD FACSCanto™ platform BD™ LSR platform BD FACSAria™ platform	PE	APC and APC-Cy™7

Flow cytometer	Reporter channel	Bead channels
BD FACSCalibur™	FL2	FL4 and FL3
BD FACSVerse™	PE	CBA Red and CBA NIR
Note: Visit bdbiosciences.com/cbasetup for setup protocols.		

- BD Falcon™ 12 × 75-mm sample acquisition tubes for a flow cytometer (Catalog No. 352008)
- 15-mL conical polypropylene tubes (BD Falcon, Catalog No. 352097), or equivalent
- FCAP Array software (Catalog No. 652099 [PC] or 645447 [Mac®])
- Microcentrifuge

Materials required for plate loader-equipped flow cytometers

- Millipore MultiScreen$_{HTS}$-BV 1.2-µm clear non-sterile filter plates [Catalog No. MSBVN1210 (10 pack) or MSBVN1250 (50 pack)]
- Millipore MultiScreen$_{HTS}$ Vacuum Manifold (Catalog No. MSVMHTS00)
- MTS 2/4 Digital Stirrer, IKA Works, VWR (Catalog No. 82006-096)
- Vacuum source
- Vacuum gauge and regulator (if not using the recommended manifold)

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Assay preparation

This section covers the following topics:

Preparing Human Flex Set Standards

Purpose of this procedure

The BD CBA Standards are lyophilized and must be reconstituted and serially diluted immediately before mixing with the Capture Beads and the Detection Reagent.

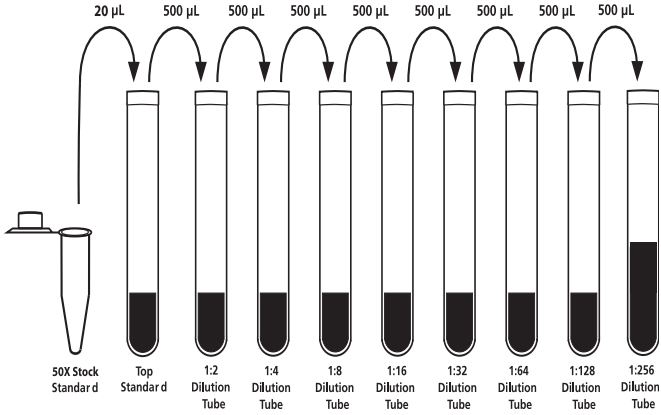
Note: You must prepare fresh standards to run with each single bead or multiplex experiment. Do not store or reuse reconstituted or diluted standards.

Procedure

To reconstitute and serially dilute the standards:

1. Open one vial of lyophilized standard from each BD CBA Human Soluble Protein Flex Set that will be tested.
2. Pool all lyophilized standard spheres into one 15-mL polypropylene tube. Label the tube "Top Standard."
3. Reconstitute the standards with 4 mL of Assay Diluent.
    a. Allow the reconstituted standard to equilibrate for at least 15 minutes at room temperature.
    b. Gently mix the reconstituted standard by pipet only. Do not vortex or mix vigorously.
4. Label eight 12 × 75-mm tubes and arrange them in the following order: 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128, and 1:256.
5. Pipette 500 µL of Assay Diluent into each of the 12 × 75-mm tubes.
6. Perform a serial dilution.
    a. Transfer 500 µL from the Top Standard to the 1:2 dilution tube and mix thoroughly by pipet only. Do not vortex.

b. Continue making serial dilutions by transferring 500 µL from the 1:2 tube to the 1:4 tube and so on to the 1:256 tube.



7. Prepare one 12 × 75-mm tube containing Assay Diluent to serve as the 0-pg/mL negative control.

Note: We recommend that the first 10 wells or tubes in the experiment be the standards. Standards should be run in order from least concentrated (0 pg/mL) to most concentrated (Top Standard) to facilitate analysis in FCAP Array software.

Concentration of standards

The approximate concentration (pg/mL) of each BD CBA Human Soluble Protein Flex Set Standard in each dilution tube is shown in the following table.

Note: See the technical data sheet for each individual assay to verify the concentration of the Top Standard.

	Dilution tube								
	Top Stand.	**1:2**	**1:4**	**1:8**	**1:16**	**1:32**	**1:64**	**1:128**	**1:256**
Protein (pg/mL)	2,500	1,250	625	312.5	156	80	40	20	10

Next step

Proceed to Mixing Human Soluble Protein Flex Set Capture Beads (page 20).

Mixing Human Soluble Protein Flex Set Capture Beads

Purpose of this procedure

The Capture Beads provided in each BD CBA Human Soluble Protein Flex Set are at a 50X concentration and must be diluted to their optimal concentration before use.

Procedure for supernatants

To mix the Capture Beads when testing supernatants:

1. Determine the number of BD CBA Human Soluble Protein Flex Sets to be used in the experiment (size of the multiplex).

2. Determine the number of tests in the experiment.

   Note: Extra tests of Capture Beads should be mixed to ensure that the necessary number of tests will be recovered from the mixed Capture Beads tube. Add an additional 2 to 3 assay tubes to the number determined.

3. Vortex each Capture Bead stock vial for at least 15 seconds to resuspend the beads thoroughly.

4. Determine the total volume of diluted beads needed for the experiment. Each tube/well requires 50 µL of the diluted beads. The total volume of diluted beads can be calculated by multiplying the number of tests (determined in step 2) by 50 µL.

   Example: 35 tests × 50 µL = 1,750 µL total bead volume

5. Determine the volume needed for each Capture Bead. Beads are supplied so that 1.0 µL = 1 test. Therefore, the required volume (µL) of beads is equal to the number of tests.

   Example: 35 tests requires 35 µL of each Capture Bead included in the assay

6. Determine the volume of Capture Bead Diluent needed to dilute the beads. Calculate the Diluent volume by subtracting the volume for each bead tested from the total volume of diluted beads needed to perform the assay. See Capture Bead and PE Detection Reagent Diluent Calculations (page 38).

   Example: 1,750 µL total volume of beads – 35 µL for each bead = volume of Capture Bead Diluent

   - if testing one analyte: 1,750 µL – (35 µL × 1) = 1,715 µL diluent
   - if testing five analytes: 1,750 µL – (35 µL × 5) = 1,575 µL diluent

7. Pipette the Capture Beads and Capture Bead Diluent into a tube labeled "Mixed Capture Beads."

Procedure for serum and plasma samples

To mix the Capture Beads when testing serum or plasma samples:

1. Determine the number of BD CBA Human Soluble Protein Flex Sets to be used in the experiment (size of the multiplex).
2. Determine the number of tests in the experiment. Beads are supplied so that 1.0 µL = 1 test. Therefore, the required volume (µL) of beads is equal to the number of tests.

   Note: Extra tests of Capture Beads should be mixed to ensure that the necessary number of tests will be recovered from the mixed Capture Beads tube. Add an additional 2 to 3 assay tubes to the number determined.

3. Vortex each Capture Bead stock vial for at least 15 seconds to resuspend the beads thoroughly.
4. Pipette the appropriate volume (determined in step 2) of each capture bead into a tube labeled Mixed Capture Beads.
5. Add 0.5 mL Wash Buffer and centrifuge at 200*g* for 5 minutes.
6. Carefully discard the supernatant by aspiration. Avoid aspirating the bead pellet.
7. Resuspend the beads in Capture Bead Diluent for Serum/Plasma to a final concentration of 50 µL/test.
8. Vortex the Capture Beads and incubate for 15 minutes at room temperature prior to use.

   Example: 35 tests × 50 µL = 1,750 µL Capture Bead Diluent for Serum/Plasma

Next step

The Capture Beads are now ready to be transferred to the assay tubes. Discard excess prepared Capture Beads. Do not store after mixing.

If you need to dilute samples having high-protein concentrations (for example, serum or plasma samples), proceed to Diluting test samples (page 23). Otherwise, proceed to Preparing Human Soluble Protein Flex Set PE Detection Reagents (page 25).

If sample dilution is not required, you can save time by proceeding directly to Performing the Human Soluble Protein Flex Set Assay (page 30). You will need to prepare the PE Detection Reagent during the first incubation step. You will also need to perform the cytometer setup procedure during one of the incubation steps.

Diluting test samples

Purpose of this procedure

The standard curve for each BD CBA Human Soluble Protein Flex Set covers a defined set of concentrations. It might be necessary to dilute test samples to ensure that their median fluorescence values fall within the range of the generated standard curve. For best results, dilute samples that are known or assumed to contain high levels of a given protein. This procedure might not be required for all samples.

Procedure

To dilute samples with known high-protein concentrations:

1. Dilute the sample by the desired dilution factor (for example, 1:10 or 1:100) using the appropriate volume of Assay Diluent.

   Serum or plasma samples must be diluted at least 1:4 before transferring the samples to the assay tubes or wells.

2. Mix sample dilutions thoroughly before transferring samples to the appropriate assay tubes containing Capture Beads.

3. To facilitate analysis in FCAP Array software, load serial diluted samples in sequential wells from most concentrated to least concentrated (eg, Sample 1 – 1:4, 1:8, 1:16; Sample 2 – 1:4, 1:8, 1:16; etc).

Next step

Proceed to Preparing Human Soluble Protein Flex Set PE Detection Reagents (page 25).

Or, you can save time by proceeding directly to Performing the Human Soluble Protein Flex Set Assay (page 30). You will need to prepare the PE Detection Reagent during the first assay incubation step. If cytometer setup is required, you will also need to perform this procedure during an assay incubation step.

Preparing Human Soluble Protein Flex Set PE Detection Reagents

Purpose of the procedure

The PE Detection Reagent provided with each BD CBA Human Soluble Protein Flex Set is a 50X bulk concentration (1 µL per test) and should be mixed with other BD CBA Human Soluble Protein Flex Set PE Detection Reagents and diluted to the optimal volume per test (50 µL per test) before adding the PE Detection Reagents to a given tube or assay well.

Note: Protect the PE Detection Reagents from exposure to direct light because they can become photobleached and will lose fluorescence intensity.

Preparing PE Detection Reagent

To prepare Human Detection Reagents:

Note: You can use the same calculations for the number of tests and volume that you used for the Capture Beads in Mixing Human Soluble Protein Flex Set Capture Beads (page 20).

1. Determine the number of BD CBA Human Soluble Protein Flex Sets to be used in the experiment (size of the multiplex).
2. Determine the number of tests to be run in the experiment. Prepare a few additional tests than necessary to ensure that there is enough material prepared for the experiment.
3. Determine the total volume of diluted PE Detection Reagent needed for the experiment. Each tube/well requires 50 µL of the diluted PE Detection Reagent. The total volume can be calculated by multiplying the number of tests (determined in step 2) by 50.

   Example: 35 tests × 50 µL = 1,750 µL total volume

4. Determine the volume needed for each PE Detection Reagent. The PE Detection Reagent is supplied so that 1.0 µL = 1 test. Therefore, the required volume (µL) of Detection Reagent is equal to the number of tests.

   Example: 35 tests requires 35 µL of each Detection Reagent included in the assay

5. Determine the volume of Detection Reagent Diluent needed to dilute the PE Detection Reagents. Calculate the Detection Reagent Diluent volume by subtracting the volume for each PE Detection Reagent tested from the total volume of diluted PE needed to perform the assay. See Capture Bead and PE Detection Reagent Diluent Calculations (page 38).

   Example: 1,750 µL total volume PE – 35 µL for each Detection Reagent = volume of Detection Reagent Diluent

   - if testing one analyte: 1,750 µL – (35 µL × 1) = 1,715 µL diluent
   - if testing five analytes: 1,750 µL – (35 µL × 5) = 1,575 µL diluent

6. Pipette the Detection Reagents and Detection Reagent Diluent into a tube labeled "Mixed PE Detection Reagents." Store at 4°C, protected from light until ready to use.

Next step

Perform cytometer setup, if necessary, using the instrument setup beads. For details on setup, go to bdbiosciences.com/cbasetup and select the appropriate flow cytometer under CBA Flex Sets: Instrument Setup.

Or, if you wish to begin staining your samples for the assay, proceed to Performing the Human Soluble Protein Flex Set Assay (page 30), and you can perform the cytometer setup procedure during one of the incubation steps.

Note: It is not necessary to set up most digital cytometers before every experiment. Templates can be created by performing the setup and saving a template with the appropriate settings for subsequent experiments. When using a template, be sure to confirm the settings by running either setup beads or an extra well/tube from the assay to ensure that the template settings are acceptable.

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Assay procedure

This section covers the following topics:

Performing the Human Soluble Protein Flex Set Assay

Before you begin

1. Prepare the standards as described in Preparing Human Flex Set Standards (page 18).
2. Mix the Capture Beads as described in Mixing Human Soluble Protein Flex Set Capture Beads (page 20).
3. If necessary, dilute the unknown samples. See Diluting test samples (page 23).
4. Prepare the Detection Reagents as described in Preparing Human Soluble Protein Flex Set PE Detection Reagents (page 25). You can also prepare these reagents during the first assay incubation.

Overview

Following the preparation and dilution of the individual assay components, transfer the standards or samples, mixed Capture Beads, and mixed PE Detection Reagents to the appropriate assay wells or tubes for incubation and analysis.

Note: Protect Capture Beads and PE Detection Reagents from direct exposure to light.

Assay procedure for plates

To prepare the standards and samples for analysis using plates:

1. Wet the filter plate by adding 100 µL of Wash Buffer to each well. To remove the excess volume, apply to a vacuum manifold. Do not exceed 10'' Hg of vacuum pressure. Aspirate for 2 to 10 seconds until wells are drained.

2. Add 50 µL of Flex Set Standard dilutions to the first 10 wells as listed in the following table.

Well label	Standard dilution	Concentration (pg/mL)
1	no standard dilution (Assay Diluent only)	0 (negative control)
2	1:256	10
3	1:128	20
4	1:62	40
5	1:32	80
6	1:16	156
7	1:8	312.5
8	1:4	625
9	1:2	1,250
10	Top Standard	2,500

3. Add 50 µL of each unknown sample to the appropriate wells.
4. Vortex the mixed Capture Beads for at least 5 seconds.
5. Add 50 µL of the mixed Capture Beads to each assay well. Mix the plate for 5 minutes at 500 rpm using a digital shaker (do not exceed 600 rpm).
6. Incubate the plate for 1 hour at room temperature.
7. Add 50 µL of the mixed PE Detection Reagent to each assay well. Mix the plate for 5 minutes at 500 rpm using a digital shaker.
8. Incubate the plate for 2 hours at room temperature.

   Note: If you have not yet performed cytometer setup, you may wish to do so during this incubation.

9. Apply the plate to the vacuum manifold and vacuum aspirate (do not exceed 10'' Hg of vacuum pressure) for 2 to 10 seconds until the wells are drained.
10. Add 150 µL of Wash Buffer to each well. Mix the plate on a digital shaker for 5 minutes at 500 rpm to resuspend the beads.
11. Proceed to sample acquisition. See Next step (page 33) for helpful information on acquisition.

Assay procedure for tubes

To prepare the standards and samples for analysis using tubes:

1. Add 50 µL of Flex Set Standard dilutions to the first 10 tubes as listed in the following table.

Tube label	Standard dilution	Concentration (pg/mL)
1	no standard dilution (Assay Diluent only)	0 (negative control)
2	1:256	10
3	1:128	20
4	1:62	40
5	1:32	80
6	1:16	156
7	1:8	312.5
8	1:4	625
9	1:2	1,250
10	Top Standard	2,500

2. Add 50 µL of each unknown sample to the appropriate assay tubes.
3. Vortex the mixed Capture Beads for at least 5 seconds.
4. Add 50 µL of the Mixed Capture Beads to each assay tube. Gently mix the tubes.

5. Incubate the tubes for 1 hour at room temperature.
6. Add 50 µL of the mixed PE Detection Reagent to each assay tube. Gently mix the tubes.
7. Incubate the tubes for 2 hours at room temperature.

   Note: If you have not yet performed cytometer setup, you may wish to do so during this incubation.

8. Add 1 mL of Wash Buffer to each assay tube and centrifuge at 200*g* for 5 minutes.
9. Carefully aspirate and discard the supernatant from each assay tube.
10. Add 300 µL of Wash Buffer to each assay tube. Vortex assay tubes briefly to resuspend the beads.

Next step

Acquire the samples on the flow cytometer. For details, go to bdbiosciences.com/cbasetup and select the appropriate flow cytometer under CBA Flex Sets: Instrument Setup.

Acquire samples on the same day they are prepared. Prolonged storage of samples, once the assay is complete, can lead to increased background and reduced sensitivity.

To facilitate the analysis of samples in the FCAP Array software, we recommend the following guidelines:

- Acquire standards from lowest (0 pg/mL) to highest (Top Standard) concentration, followed by the test samples.
- If running sample dilutions, acquire sequentially starting with the most concentrated sample.
- Store all FCS files (standards and samples) in a single folder.

Data analysis

How to analyze data

Analyze data using FCAP Array software. For instructions on analysis, go to bdbiosciences.com/cbasetup and see the *FCAP Array Software User's Guide*.

When analyzing the BD CBA Human Soluble Protein assay data with FCAP Array software and choosing a curve fitting option, try both 4 Parameter and 5 Parameter Logistic, and select the one that results in the best curve fit (highest R^2 value).

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Reference

This section covers the following topics:

Troubleshooting

Recommended actions

These are the actions we recommend taking if you encounter the following problems.

Note: For best performance, vortex samples immediately before acquiring on a flow cytometer.

Problem	Recommended action
Poor standard curves	If there is no change in signal above background across the entire standard curve range, ensure that all of the components (Capture Beads, Detection Reagent, and standard) were added to each tube.
	If the curve is relatively flat and then increases at higher concentrations but not to the expected levels, make sure standards are not being vortexed or vigorously mixed while being reconstituted. The best approach is to allow the standards to equilibrate for 15 minutes in Assay Diluent prior to mixing. Mix by gently pipetting several times.
	Check that all components have been properly prepared and stored. Use freshly reconstituted standards. Ensure that incubation times are of proper length and that the assay did not sit for a prolonged period of time after the wash step.
Low event count	The beads can aggregate. Thoroughly vortex individual Capture Bead bulk vials prior to preparation of the master bead mix and vortex the master bead mix prior to dispensing into the individual assay wells. Thoroughly shake plates or vortex sample tubes prior to acquisition.
	Ensure that the stopping rule, singlet gate, and thresholds are set correctly.
	Ensure that the vacuum is not too strong and that filter membranes are not compromised (filter plates). Avoid aspiration of beads during the wash step (tubes).
Variation between duplicate samples	Vortex Capture Beads before pipetting. Beads can aggregate.

Problem	Recommended action
Little or no detection of protein in samples	Samples might be too dilute. Try various sample dilutions.
All samples are positive or above the high standard median fluorescence value	Samples might be too concentrated. Try various sample dilutions.
High background	Remove excess PE Detection Reagent by increasing the number of wash steps, since the background might be due to non-specific binding.
	Background may be produced by precipitated buffers. Check for visible precipitate and filter through a 0.2-µm filter, if necessary.
Sample dilution	We recommend diluting serum and plasma samples at least 1:4 because spike recoveries are generally better, suggesting that factors might be present at lower dilutions that inhibit the binding kinetics of the assay. If using the filter plate protocol, diluting the samples also prevents clogging of the filter membrane, which can lead to insufficient washing and high background. If using a BD FACSCalibur cytometer, diluting the samples along with adding additional standard dilutions ensures that sample MFIs fall on the linear portion of the curve and prevents spillover of excessive PE signal into the FL3 channel, which can cause gating issues during software analysis.
Sample storage	Cytokines in general are quite labile and will degrade over time even when stored frozen at –70°C. Samples can usually be stored in single-use aliquots for 3–6 months. Sample storage strategies should be determined empirically prior to making them standard practice.

Problem	Recommended action
Biohazardous samples	It is possible to treat samples with 1% paraformaldehyde before analyzing on the flow cytometer. This might affect assay performance and should be validated. The antibody pairs are optimized for detection of native protein, so fixation should be attempted only after the samples have been incubated with the capture and detection reagents.
Anticoagulant for plasma samples	Only EDTA plasma samples have been verified by BD Biosciences.
Clogged filter plate	Serum and plasma proteins can settle and clog the membrane during incubation. Dilute samples further or perform assay incubations in a standard polystyrene U-bottom plate (Catalog No. 353910) and transfer to the filter plate immediately prior to aspiration. Resuspend the beads well prior to transfer.

Capture Bead and PE Detection Reagent Diluent Calculations

How to calculate Calculate the Diluent volume by subtracting the volume for each bead tested from the total volume of diluted beads needed to perform the assay. The following table lists the appropriate volumes.

No. of Flex Sets to be used	Volume per test			
	Each Capture Bead or Detection Reagent	Total Capture Bead	Capture Bead or Detection Reagent Diluent	Mixed Capture Beads or Detection Reagent
1	1 µL	1 µL	49 µL	50 µL
2	1 µL	2 µL	48 µL	50 µL
3	1 µL	3 µL	47 µL	50 µL
4	1 µL	4 µL	46 µL	50 µL
5	1 µL	5 µL	45 µL	50 µL
6	1 µL	6 µL	44 µL	50 µL
7	1 µL	7 µL	43 µL	50 µL
8	1 µL	8 µL	42 µL	50 µL
9	1 µL	9 µL	41 µL	50 µL
10	1 µL	10 µL	40 µL	50 µL
11	1 µL	11 µL	39 µL	50 µL
12	1 µL	12 µL	38 µL	50 µL
13	1 µL	13 µL	37 µL	50 µL
14	1 µL	14 µL	36 µL	50 µL
15	1 µL	15 µL	35 µL	50 µL
16	1 µL	16 µL	34 µL	50 µL
17	1 µL	17 µL	33 µL	50 µL
18	1 µL	18 µL	32 µL	50 µL
19	1 µL	19 µL	31 µL	50 µL
20	1 µL	20 µL	30 µL	50 µL
21	1 µL	21 µL	29 µL	50 µL
22	1 µL	22 µL	28 µL	50 µL
23	1 µL	23 µL	27 µL	50 µL
24	1 µL	24 µL	26 µL	50 µL
25	1 µL	25 µL	25 µL	50 µL
26	1 µL	26 µL	24 µL	50 µL
27	1 µL	27 µL	23 µL	50 µL
28	1 µL	28 µL	22 µL	50 µL
29	1 µL	29 µL	21 µL	50 µL
30	1 µL	30 µL	20 µL	50 µL

References

General related publications

1. Bishop JE, Davis KA. A flow cytometric immunoassay for β2-microglobulin in whole blood. *J Immunol Meth*. 1997;210:79-87.
2. Camilla C, Defoort JP, Delaage M, et al. A new flow cytometry-based multi-assay system: Application to cytokine immunoassays. *Cytometry Suppl*. 1998;8:132.
3. Carson R, Vignali D. Simultaneous quantitation of fifteen cytokines using a multiplexed flow cytometric assay. *J Immunol Meth*. 1999;227:41-52.
4. Chen R, Lowe L, Wilson JD, et al. Simultaneous quantification of six human cytokines in a single sample using microparticle-based flow cytometric technology. *Clin Chem*. 1999;45:1693-1694.
5. Kricka LJ. Simultaneous multianalyte immunoassays. In: Immunoassay. Diamandis EP, Christopoulos TK, eds. Academic Press. 1996:389-404.
6. Lund-Johansen FK, Davis KJ, Bishop J, Malefyt RW. Flow cytometric analysis of immunoprecipitates: High-throughput analysis of protein phosphorylation and protein-protein interactions. *Cytometry*. 2000;39:250-259.
7. McHugh TM. Flow microsphere immunoassay for the quantitative and simultaneous detection of multiple soluble analytes. *Methods Cell Biol*. 1994;42:575-595.
8. Oliver KG, Kettman JR, Fulton RJ. Multiplexed analysis of human cytokines by use of the FlowMetrix system. *Clin Chem*. 1998;44:2057-2060.

9. Cook EB, Stahl JL, Lowe L, et al. Simultaneous measurement of six cytokines in a single sample of human tears using microparticle-based flow cytometry: allergics vs. non-allergics. *J Immunol Meth*. 2001;254:109-118.

10. Dotti G, Savoldo B, Takahashi S, et al. Adenovector-induced expression of human-CD40-ligand (hCD40L) by multiple myeloma cells: A model for immunotherapy. *Exp Hematol*. 2001;29:952-961.

Product-related publications

1. Arnulf B, Lecourt S, Soulier J, et al. Phenotypic and functional characterization of bone marrow mesenchymal stem cells derived from patients with multiple myeloma. *Leukemia*. 2007;21:158-163.

2. Dionne SO, Podany AB, Ruiz YW, Ampel NM, Galgiani JN, Lake DF. Spherules derived from Coccidioides posadasii promote human dendritic cell maturation and activation. *Infect Immun*. 2006;74:2415-2422.

3. Herberth G, Daegelmann C, Weber A, et al. Association of neuropeptides with Th1/Th2 balance and allergic sensitization in children. *Clin Exp Allergy*. 2006;36:1408-1416.

4. Jury EC, Isenberg DA, Mauri C, Ehrenstein MR. Atorvastatin restores Lck expression and lipid raft-associated signaling in T cells from patients with systemic lupus erythematosus. *J Immunol*. 2006;177:7416-7422.

5. Kobasa D, Jones SM, Shinya K, et al. Aberrant innate immune response in lethal infection of macaques with the 1918 influenza virus. *Nature*. 2007;445:319-323.

6. Kuwano Y, Fujimoto M, Watanabe R, et al. Serum chemokine profiles in patients with alopecia areata. *Br J Dermatol*. 2007;157:466-473.

7. Martinelli E, Cicala C, Van Ryk D, et al. HIV-1 gp120 inhibits TLR9-mediated activation and IFN-α secretion in plasmacytoid dendritic cells. *Proc Natl Acad Sci USA*. 2007;104:3396-3401.

8. McKinnon LR, Ball TB, Wachihi C, et al. Epitope Cross-Reactivity Frequency differs between central and effector memory HIV-specific CD8+ T cells. *J Immunol*. 2007;178:3750-3756.

9. Potapova IA, Gaudette GR, Brink PR, et al. Mesenchymal stem cells support migration, extracellular matrix invasion, proliferation, and survival of endothelial cells in vitro. *Stem Cells*. 2007;25:1761-1768.

10. Shin JJ, Glickstein LJ, Steere AC. High levels of inflammatory chemokines and cytokines in joint fluid and synovial tissue throughout the course of antibiotic-refractory Lyme arthritis. *Arthritis Rheum*. 2007;56:1325-1335.

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