BD™ Cytometric Bead Array

Mouse/Rat Soluble Protein Master Buffer Kit

Instruction Manual

Cat. No. 558266 100 Tests

Cat. No. 558267 500 Tests

BD

Kit Contents

558266, 100 tests

(Store the following items at 4°C)

Assay Diluent: 1 bottle, 30 mL

Capture Bead Diluent: 1 bottle, 5 mL

Detection Reagent Diluent: 1 bottle, 5 mL

Wash Buffer: 1 bottle, 130 mL

Instrument Setup Bead A1: 1 vial, 0.25 mL

Instrument Setup Bead A9: 1 vial, 0.25 mL

Instrument Setup Bead F1: 1 vial, 1.0 mL

Instrument Setup Bead F9: 1 vial, 0.25 mL

PE Instrument Setup Bead F1: 1 vial, 0.25 mL

PE* Positive Control Detector: 1 vial, 0.5 mL

Kit Contents

558267, 500 tests

(Store the following items at 4°C)

Assay Diluent: 1 bottle, 150 mL

Capture Bead Diluent: 1 bottle, 30 mL

Detection Reagent Diluent: 1 bottle, 30 mL

Wash Buffer: 1 bottle, 650 mL

Instrument Setup Bead A1: 1 vial, 0.25 mL

Instrument Setup Bead A9: 1 vial, 0.25 mL

Instrument Setup Bead F1: 1 vial, 1.0 mL

Instrument Setup Bead F9: 1 vial, 0.25 mL

PE Instrument Setup Bead F1: 1 vial, 0.25 mL

PE* Positive Control Detector: 1 vial, 0.5 mL

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Introduction

Flow cytometry is an analysis tool that allows for the discrimination of different particles on the basis of size and fluorescence. The BD™ Cytometric Bead Array (CBA) Mouse or Rat Soluble Protein Flex Sets and Mouse/Rat Soluble Protein Master Buffer Kits employ particles with discrete fluorescence intensities to detect soluble analytes. The BD CBA is combined with flow cytometry to create a powerful particle-based immunoassay.

The BD CBA Mouse/Rat Soluble Protein Flex Set system uses the sensitivity of amplified fluorescence detection by flow cytometry to measure a soluble analyte. Each bead in a BD CBA provides a capture surface for a specific protein and is analogous to an individually coated well in an ELISA plate. The BD CBA Mouse or Rat Soluble Protein Flex Set capture bead is in suspension to allow for the detection of an analyte in a small sample volume. The combined advantages of the broad dynamic range of fluorescent detection via flow cytometry and the efficient capturing of multiple analytes via suspended particles enable the BD CBA Flex Set system to use fewer sample dilutions and to obtain the value of an unknown in substantially less time (compared to conventional ELISA).

The BD CBA Mouse/Rat Soluble Protein Master Buffer Kit contains all of the supporting reagents necessary to perform an assay using a BD CBA Mouse or Rat Soluble Protein Flex Set. The buffers and instrument setup reagents provided in this kit have been optimized for use on BD flow cytometers.

Note: The BD CBA Mouse/Rat Soluble Protein Master Buffer Kit should not be used with any non-Mouse or Rat Soluble Protein BD CBA Flex Sets. BD CBA Mouse and Rat Soluble Protein Flex Set Assays may not be performed in the same experiment well.

Principle of the Test

A BD CBA Mouse or Rat 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 resolvable in the NIR and Red channels of a BD FACSArray™ bioanalyzer or the FL3 and FL4 channels of a BD FACSCalibur™ flow cytometer.

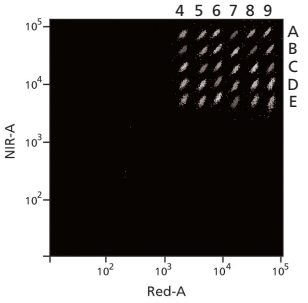


Figure 1

Each bead population is given an alpha-numeric position designation indicating its position relative to other beads in the BD CBA Flex Set system. Beads with different positions can be combined in assays to create a multiplex assay. In a BD CBA Flex Set assay the capture bead, PE-conjugated detection reagent, and standard or test samples are incubated together to form sandwich complexes. Following acquisition of sample data using the flow cytometer, the sample results are generated in graphical and tabular formats using the FCAP Array™ software. The BD CBA Mouse/Rat Soluble Protein Master Buffer Kit provides sufficient reagents for the analysis of 100 samples and 10 instrument setup procedures (Cat. No. 558266) or 500 samples and 10 instrument setup procedures (Cat. No. 558267).

Advantages

The BD CBA Mouse/Rat Soluble Protein Flex Set system provides several advantages when compared with conventional ELISA:

- The BD CBA Mouse or Rat Soluble Protein Flex Set assays allow for multiplexed analysis of multiple proteins from a single sample.
- The BD CBA Mouse or Rat Soluble Protein Flex Set assays have a wider dynamic range than conventional ELISAs.

Limitations

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

Quantitative results or protein levels for the same sample or recombinant protein run in ELISA and BD CBA assays may 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 Mouse or Rat Soluble Protein Flex Set assays are multiplexed, it is possible that the background (MFI of the 0 pg/mL standard point) may increase and the overall assay signals of other standard points may be reduced. This can result in lower dynamic range or loss in sensitivity in some assays. This effect may be greater as more assays are added to the multiplex.

For assays that will be acquired on a BD FACSCalibur flow cytometry instrument, it is recommended that additional dilutions of the standard be prepared (ie, 1:512 and 1:1024) as it is possible that in multiplex experiments containing a large number of assays the Top Standard, 1:2, and 1:4 standard dilutions will not be analyzable by the FCAP Array software. In those cases, the Top Standard, 1:2, and 1:4 standard dilutions can be run in the experiment but may need to be excluded from the final analysis in the FCAP Array software. Please see the ***BD FACSCalibur setup manual*** for further limitations.

Warnings and Precautions

All components of this kit contain phosphate buffered solution containing protein* and 0.09% sodium azide. Sodium azide yields a highly toxic hydrazoic acid under acidic conditions. Avoid exposure to skin and eyes, ingestion, and contact with heat, acids, and metals. Wash exposed skin with soap and water Flush eyes with water. Dilute azide compounds in running water before discharging to avoid accumulation of potentially explosive deposits in plumbing.

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

Materials Required but not Provided

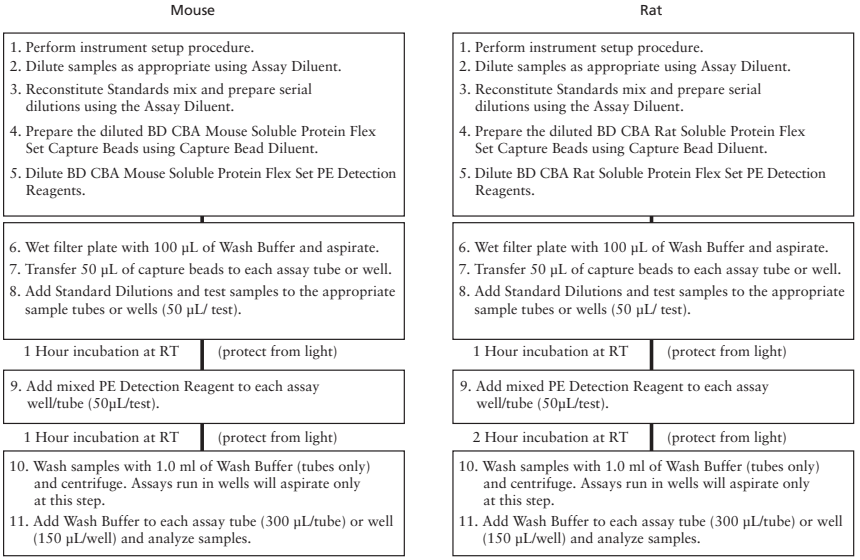
In addition to the reagents provided in the BD CBA Mouse/Rat Soluble Protein Master Buffer Kit the following items are also required:

- BD CBA Mouse/Rat Soluble Protein Flex Sets.
- 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. Refer to ***Appendix,*** Table 2 for examples of compatible instrument platforms.
- 12 × 75 mm sample acquisition tubes for a flow cytometer (eg, BD Falcon™ Cat. No. 352008).
- FCAP Array software (Cat. No. 641488).
- BD CaliBRITE™ 3 beads, (Cat. No. 340486) to run experiments on a BD FACSCalibur.
- BD CaliBRITE APC beads, (Cat. No. 340487) to run experiments on a BD FACSCalibur.
- BD FACSComp™ software to run experiments on a BD FACSCalibur.
- Microcentrifuge.

Required for Plate-loader-equipped Flow Cytometers

- Millipore MultiScreen$_{HTS}$-BV 1.2 µm Clear non-sterile filter plates, [Cat. No. MSBVN1210 (10 pack) or MSBVN1250 (50 pack)].
- Millipore MultiScreen$_{HTS}$ Vacuum Manifold, (Cat. No. MSVMHTS00).
- MTS 2/4 Digital Stirrer, IKA Works, VWR, (Cat. No. 82006-096).
- Standard microtiter plate for BD FACSArray Bioanalyzer Setup (BD Falcon Cat. No. 353910).
- Vacuum source.
- Vacuum gauge and regulator (if not using recommended manifold).

Overview: BD CBA Mouse/Rat Soluble Protein Flex Set Assay Procedure



Perform Instrument Setup

In order to ensure that the flow cytometer is performing optimally, perform the instrument setup procedure prior to preparing the Flex Set Assay. Refer to the appropriate flow cytometry instrument setup manual included in this kit for instructions on how to setup your instrument.

Preparation of Test Samples

The standard curve for each BD CBA Mouse/Rat Soluble Flex Set covers a defined set of concentrations. It may be necessary to dilute test samples to ensure that their mean fluorescence values fall within the limits or range of the generated standard curve. For best results, samples that are known or assumed to contain high levels of a given protein should be diluted as described below.

1. Dilute test sample by the desired dilution factor (eg, 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 tubes/wells containing the Capture Beads.
3. In order 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).

Preparation of BD CBA Mouse/Rat Soluble Protein Flex Set Standards

For each single bead or multiplex assay a standard curve will need to be prepared. The protocol below indicates how standards should be mixed and diluted for use in a BD CBA Mouse/Rat Soluble Flex Set.

1. Remove one lyophilized standard vial from each BD CBA Mouse/Rat Soluble Flex Set that will be tested.
2. Open each vial of lyophilized standard.
3. Pool all lyophilized standard spheres into one tube (Recommended 15 mL Conical Tube, BD Falcon Cat. No. 352097). Label tube "Top Standard".
4. Reconstitute the standards with 4.0 mL of Assay Diluent. Allow the reconstituted standard to equilibrate for at least 15 minutes before making dilution. **Mix reconstituted protein by pipette only. Do not vortex or mix vigorously.**
5. Label 12 × 75 mm tubes (BD Falcon Cat. No. 352008) and arrange them in the following order: 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128 and 1:256.
6. Pipette 500 µL of Assay Diluent to each of the remaining tubes.
7. Perform a serial dilution by transferring 500 µL from the Top Standard to the 1:2 dilution tube and mix thoroughly. 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 and mix thoroughly (see ***Figure 2***). **Mix by pipette only, do not vortex.** Prepare one tube containing Assay Diluent to serve as the 0 pg/mL negative control.
8. It is recommended that the first ten 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). See ***Appendix***, Table 3.

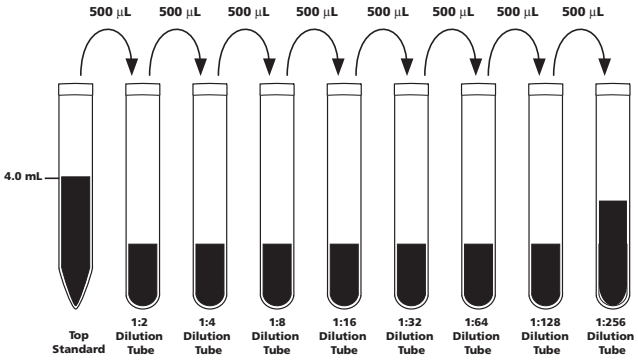


Figure 2. Preparation of BD CBA Mouse or Rat Soluble Protein Flex Set Standard Dilutions

The typical concentration (pg/mL) of each BD CBA Mouse or Rat Soluble Protein Flex Set Standard in each dilution tube is shown in Table 1. Refer to the BD CBA Flex Set technical data sheet for details on the bulk (10×) concentration of the standard for a given assay.

Table 1. BD CBA Mouse/Rat Soluble Protein Flex Set Standard concentrations after dilution

BD CBA Mouse Soluble Protein Flex Set	Top Standard	1:2 Dilution Tube	1:4 Dilution Tube	1:8 Dilution Tube	1:16 Dilution Tube	1:32 Dilution Tube	1:64 Dilution Tube	1:128 Dilution Tube	1:256 Dilution Tube
Protein (pg/ml)	2500	1250	625	312.5	156	80	40	20	10

BD CBA Rat Soluble Protein Flex Set	Top Standard	1:2 Dilution Tube	1:4 Dilution Tube	1:8 Dilution Tube	1:16 Dilution Tube	1:32 Dilution Tube	1:64 Dilution Tube	1:128 Dilution Tube	1:256 Dilution Tube
Protein (pg/ml)	10,000	5,000	2,500	1,250	625	312.5	156	80	40

Preparation of BD CBA Mouse or Rat Soluble Protein Flex Set Capture Beads

The Capture Beads provided in each BD CBA Mouse/Rat Soluble Flex Set are at a 50× concentration and must be diluted to their optimal concentration before adding to a given assay tube or assay well.

1. Determine the number of BD CBA Mouse or Rat Soluble Protein Flex Sets to be used in the experiment (size of the multiplex).
2. Determine the number of tests in the experiment. It is recommended that the user prepare a few additional tests than they will use in the experiment to ensure that there is enough material prepared for the experiment.
3. Vortex each Capture Bead stock vial for at least 15 seconds to resuspend 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 above) by 50 µL.
   - eg, 35 tests × 50 µL = 1750 µL total volume of diluted beads.
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.
   - eg, 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. The volume of Capture Bead Diluent can be calculated by subtracting the volume for each bead tested from the total volume of diluted beads needed to perform the assay. Refer to Table 4 in the ***Appendix*** for more examples of this calculation.
   - eg, 1750 µL total volume of diluted beads - 35 µL for each bead = volume of Capture Bead Diluent.
   - eg, if testing one analyte: 1750 µL – (35 µL × 1) = 1715 µL diluent.
   - eg, if testing 5 analytes: 1750 µL – (35 µL × 5) = 1575 µL diluent.
7. Pipette the Capture Beads and Capture Bead Diluent into a tube labeled Mixed Capture Beads.

Preparation of BD CBA Mouse or Rat Soluble Protein Flex Set PE Detection Reagents

The PE Detection Reagent provided with each BD CBA Mouse or Rat Soluble Protein Flex Set is a 50× bulk (1 µL/test) and should be mixed with other BD CBA Mouse or Rat Soluble Protein Flex Set PE Detection Reagent and diluted to their optimal volume per test (50 µL/test) before adding the PE Detection Reagents to a given tube or assay well. The calculations below should be the same as in the previous section.

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

1. Determine the number of BD CBA Mouse or Rat Soluble Protein Flex Sets to be used in each assay tube or well in the experiment (size of the multiplex).
2. Determine the number of assay tests to be run in the experiment. It is recommended that the user prepare a few additional tests than they will use in the experiment 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 of diluted PE can be calculated by multiplying the number of tests (determined above).
   - eg, 35 tests × 50 µL = 1750 µL total volume of diluted PE.
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 PE Detection Reagent is equal to the number of tests.
   - eg, 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. The volume of Detection Reagent Diluent can be calculated by subtracting the volume for each PE Detection Reagent tested from the total volume of diluted PE needed. Refer to Table 4 in the ***Appendix*** for more examples.
   - eg, 1750 µL total volume diluted PE - 35 µL for each Detection Reagent = volume of Detection Reagent Diluent.
   - eg, if testing one analyte: 1750 µL – (35 µL × 1) = 1715 µL diluent.
   - eg, if testing 5 analytes: 1750 µL – (35 µL × 5) = 1575 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.

BD CBA Mouse or Rat Soluble Protein Flex Set Assay Procedure

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.

For Plates:

1. Prepare all reagents as described in previous sections before starting the experiment.
2. Pre-wet the plate by adding 100 µL of Wash Buffer to each well. To remove the excess volume, apply to vacuum manifold. Do not exceed 10" Hg of vacuum pressure. Aspirate until wells are drained (2 - 10 seconds).
3. Vortex the Mixed Capture Beads for at least 5 seconds. Add 50 µL of the Mixed Capture Beads to each assay well.
4. Add 50 µL of Standard or sample to the assay wells.

   Note: See ***Appendix***, table 3 for a list of essential control wells to be run in each experiment.
5. Mix the plate for 5 minutes using a digital shaker at 500 RPM (do not exceed 600 RPM) and incubate plate for 1 hour at RT.
6. Add 50 µL of the Mixed PE Detection Reagent to each assay well.
7. Mix the plate for 5 minutes using a digital shaker at 500 RPM and incubate plate at RT for 1 hour for mouse assays and 2 hours for rat assays.
8. Apply the plate to the vacuum manifold and vacuum aspirate (Do not exceed 10" Hg of vacuum pressure) until wells are drained (2 – 10 seconds).
9. Add 150 µL of Wash Buffer to each assay well. Shake microwell plate on a digital shaker at 500 RPM for 5 minutes to resuspend beads.
10. Begin analyzing samples on a flow cytometer. Proceed to the appropriate flow cytometry instrument instruction manual for acquiring the BD CBA Flex Sets.

    Note: It is best to analyze samples on the day of the experiment. Prolonged storage of samples, once the assay is complete, can lead to increased background and reduced sensitivity.

For Tubes:

1. Prepare all reagents as described in previous sections before starting the experiment.
2. Vortex the Mixed Capture Beads for at least 5 seconds. Add 50 µL of the Mixed Capture Beads to each assay tube.
3. Add 50 µL of Standard or sample to each assay tube.
4. Mix assay tubes gently and incubate for 1 hour at RT.
5. Add 50 µL of the Mixed PE Detection Reagent to each assay tube.
6. Mix assay tubes gently and incubate at RT for 1 hour for mouse assays and 2 hours for rat assays.
7. Add 1.0 mL of Wash Buffer to each assay tube and centrifuge at 200 × *g* for 5 minutes.
8. Carefully aspirate and discard the supernatant from each assay tube.
9. Add 300 µL of Wash Buffer to each assay tube. Vortex assay tubes briefly to resuspend beads.
10. Begin analyzing samples on a flow cytometer. It is recommended that each tube be vortexed briefly before analyzing on the flow cytometer. Proceed to the appropriate flow cytometry instrument instruction manual for acquiring the BD CBA Flex Sets.

    Note: It is best to analyze samples on the day of the experiment. Prolonged storage of samples, once the assay is complete, can lead to increased background and reduced sensitivity.

Troubleshooting Tips

Problem	Suggested Solution
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 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 were 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 precipitate, thoroughly vortex individual capture bead bulk vials prior to preparation of master bead mix and vortex the master bead mix prior to dispensing into the individual assay wells. Thoroughly shake plate or vortex sample tubes prior to acquisition.
	Ensure that the stopping rule, singlet gate, and thresholds are set correctly.
	Ensure that vacuum is not too strong and that filter membranes are not compromised (filter plates). Avoid aspiration of beads during wash step (tubes).
Variation between duplicate samples	Vortex capture beads before pipetting. Beads can aggregate.
Little or no detection of protein in samples	Samples may be too dilute. Try various sample dilutions.
All samples are positive or above the high standard mean fluorescence value	Samples may be too concentrated Try various sample dilutions.
High background	Remove excess PE detection reagent by increasing the number of wash steps as the background may 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	It is recommended to dilute serum and plasma samples at least 1:4 because spike recoveries are generally better, suggesting that factors may 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, 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.
Biohazardous samples	It is possible to treat samples with 1% paraformaldehyde before analyzing on the flow cytometer. This may affect assay performance and should be validated by the end user. The antibody pairs are optimized for detection of native protein so fixation should only be attempted after the samples have been incubated with the capture and detection reagents.
Anticoagulant for plasma samples	Only EDTA plasma samples have been validated 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 (BD 353910) and transfer to the filter plate immediately prior to aspiration. Resuspend the beads well prior to transfer.

References

1. Bishop, J.E. and K.A. Davis. 1997. A flow cytometric immunoassay for β2-microglobulin in whole blood. *J. Immunol. Methods.* 210:79-87.
2. Camilla C., J.P. Defoort, M. Delaage, R. Auer, J. Quintana, T. Lary, R. Hamelik, S. Prato, B. Casano, M. Martin and V. Fert. 1998. A new flow cytometry-based multi-assay system. 1. Application to cytokine immunoassays. *Cytometry Suppl.* 8:132.
3. Carson, R., and D. Vignali. 1999. Simultaneous quantitation of fifteen cytokines using a multiplexed flow cytometric assay. *J. Immunol. Methods.* 227: 41-52.
4. Chen, R., L. Lowe, J.D. Wilson, E. Crowther, K. Tzeggai, J.E. Bishop and R. Varro. 1999. Simultaneous quantification of six human cytokines in a single sample using microparticle-based flow cytometric technology. *Clin. Chem*. 9:1693-1694.
5. Kricka, LJ. 1996. Simultaneous multianalyte immunoassays. In *Immunoassay*. Diamandis, E.P. and T.K. Christopoulos, eds. *Academic Press*. pp.389-404.
6. Lund-Johansen, F., K. Davis, J. Bishop and R. de W. Malefyt. 2000. Flow cytometric analysis of immunoprecipitates: High-throughput analysis of protein phosphorylation and protein-protein interactions. *Cytometry.* 39:250-259.
7. McHugh, T.M. 1994. Flow microsphere immunoassay for the quantitative and simultaneous detection of multiple soluble analytes. *Methods in Cell Biology.* 42:575-595.
8. Oliver, K.G., J.R. Kettman and R.J. Fulton. 1998. Multiplexed analysis of human cytokines by use of the FlowMetrix system. *Clin. Chem.* 44:2057-60.
9. Stall, A., Q. Sun, R. Varro, L. Lowe, E. Crowther, B. Abrams, J. Bishop, and K. Davis. 1998. *A single tube flow cytometric multibead assay for isotyping mouse monoclonal antibodies*. Abstract LB77. Experimental Biology Meeting 1998 (late-breaking abstracts).
10. Cook, E.B., J.L. Stahl, L. Lowe, R. Chen, E. Morgan, J. Wilson, R. Varro, A. Chan, F.M. Graziano, and N.P. Barney. 2001. Simultaneous measurement of six cytokines in a single sample of human tears using microparticle-based flow cytometry: allergics vs. non-allergics. *J. Immunol. Methods.* 254: 109-118.
11. Dotti, G., B. Salvoldo, S. Takahashi, T. Goltsova, M. Brown, D. Rill, C. Rooney, and M. Brenner. 2001. Adenovector-induced expression of human-CD40-ligand (hCD40L) by multiple myeloma cells: A model for immunotherapy. *Exp. Hematol*. 29: 952 - 961.

Appendix

Table 2. Recommended Instrument Platforms

Flow Cytometer	Reporter Channel	Bead Channel
BD FACSArray	Yellow	Red and NIR
BD FACSCanto™ II* BD™ LSR II* BD FACSAria™*	PE	APC and APC-Cy7
BD FACSCalibur	FL2	FL4 and FL3

*Please visit **bdbiosciences.com/flexset** for setup protocol.

Table 3. Essential control assay tubes

Tube No.	Reagents (All reagent volumes are 50 µL)
1 Negative Control	Capture Beads, Assay Diluent, PE Detection Reagent
2 10 or 40 pg/mL Standard	Capture Beads, Standard 1:256 Dilution, PE Detection Reagent
3 20 or 80 pg/mL Standard	Capture Beads, Standard 1:128 Dilution, PE Detection Reagent
4 40 or 156 pg/mL Standard	Capture Beads, Standard 1:64 Dilution, PE Detection Reagent
5 80 or 312.5 pg/mL Standard	Capture Beads, Standard 1:32 Dilution, PE Detection Reagent
6 156 or 625 pg/mL Standard	Capture Beads, Standard 1:16 Dilution, PE Detection Reagent
7 312.5 or 1250 pg/mL Standard	Capture Beads, Standard 1:8 Dilution, PE Detection Reagent
8 625 or 2500 pg/mL Standard	Capture Beads, Standard 1:4 Dilution, PE Detection Reagent
9 1250 or 5000 pg/mL Standard	Capture Beads, Standard 1:2 Dilution, PE Detection Reagent
10 2500 or 10,000 pg/mL Standard	Capture Beads, Standard "Top Standard", PE Detection Reagent

Table 4. Capture Bead and Detection Reagent Diluent Calculations

No. of Flex Sets to be used	Volume of each Capture Bead or PE Detection Reagent/test	Total Capture Bead volume/test	Volume of Capture Bead or Detection Reagent Diluent/test	Total volume of mixed Capture Beads or PE Detection Reagents/test
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
31	1 µL	31 µL	19 µL	50 µL
32	1 µL	32 µL	18 µL	50 µL
33	1 µL	33 µL	17 µL	50 µL
34	1 µL	34 µL	16 µL	50 µL
35	1 µL	35 µL	15 µL	50 µL
36	1 µL	36 µL	14 µL	50 µL
37	1 µL	37 µL	13 µL	50 µL
38	1 µL	38 µL	12 µL	50 µL
39	1 µL	39 µL	11 µL	50 µL
40	1 µL	40 µL	10 µL	50 µL
41	1 µL	41 µL	9 µL	50 µL
42	1 µL	42 µL	8 µL	50 µL
43	1 µL	43 µL	7 µL	50 µL
44	1 µL	44 µL	6 µL	50 µL
45	1 µL	45 µL	5 µL	50 µL
46	1 µL	46 µL	4 µL	50 µL
47	1 µL	47 µL	3 µL	50 µL
48	1 µL	48 µL	2 µL	50 µL
49	1 µL	49 µL	1 µL	50 µL
50	1 µL	50 µL	0 µL	50 µL

United States
877.232.8995

Canada
888.259.0187

Europe
32.53.720.550

Japan
0120.8555.90

Asia/Pacific
65.6861.0633

Latin America/Caribbean
55.11.5185.9645

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