

Guide to Using
BD Cytometric Bead Array
(CBA) Kits with the
BD FACSCalibur System
Instruction Manual



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Regulatory information

BD flow cytometers are class I (1) laser products.

For Research Use Only. Not for use in diagnostic or therapeutic procedures.

History

Revision	Date	Change made
23-12406-00 Rev. 01	10/2010	New document

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About this guide

This section covers the following topics:

- [Purpose of this guide \(page 6\)](#)
- [How this guide relates to other BD CBA guides \(page 7\)](#)
- [Obtaining the templates \(page 8\)](#)

Purpose of this guide

Who should read this guide This guide provides setup and acquisition instructions for using a BD FACSCalibur™ flow cytometer (single or dual laser) to acquire data obtained with a BD™ Cytometric Bead Array (CBA) kit.

Software This guide assumes you are using BD CellQuest™ or BD CellQuest Pro software for acquisition.

Applicable BD CBA kits The procedures in this guide are designed for BD CBA kits that contain either a PE detection reagent or a FITC detection reagent, and single-color capture beads.

Examples of such BD CBA kits are listed in the following table.

BD CBA kit name	Catalog number
Human Anaphylatoxin Kit	561418
Human Chemokine Kit	552990
Human Inflammatory Cytokines Kit	551811
Human Th1/Th2 Cytokine Kit	550749
Human Th1/Th2 Cytokine Kit II	551809
Human Th1/Th2/Th17 Cytokine Kit	560484
Mouse Immunoglobulin Isotyping Kit	550026
Mouse Inflammation Kit	552364
Mouse Th1/Th2 Cytokine Kit	551287
Mouse Th1/Th2/Th17 Cytokine Kit	560485
Non-Human Primate Th1/Th2 Cytokine Kit	557800

How this guide relates to other BD CBA guides

Where to find instructions

The workflow for a BD CBA assay consists of four basic stages. The following table describes where to find instructions for each stage if you are using the BD FACSCalibur flow cytometer.

For information about...	See...
1. Preparing reagents, standards, and samples for use in the assay	The instruction manual that came with your BD CBA kit
2. Setting up the cytometer using BD CellQuest software	This guide
3. Acquiring data using BD CellQuest software	This guide
4. Analyzing data	<i>Guide to Analyzing Data from BD Cytometric Bead Array (CBA) Kits Using FCAP Array Software</i> , found at bdbiosciences.com/cbasetup

Obtaining the templates

About the template

Setup and acquisition templates for both single- and dual-laser BD FACSCalibur systems are available at bdbiosciences.com/cbasetup. You need to download these templates only once and then can use them as many times as necessary.

Procedure

To obtain the appropriate template:

1. Go to bdbiosciences.com/cbasetup.
 2. Download the appropriate template:
 - BD FACSCalibur Single-Laser Setup Template
 - BD FACSCalibur Single-Laser Acquisition Template
 - BD FACSCalibur Dual-Laser Setup Template
 - BD FACSCalibur Dual-Laser Acquisition Template
 3. Unzip and save the file.
-

Next step

Proceed to either [Single-laser instrument setup \(page 11\)](#) or [Dual-laser instrument setup \(page 15\)](#).

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Cytometer setup

This section covers the following topics:

- [Requirements for assay setup \(page 10\)](#)
- [Single-laser instrument setup \(page 11\)](#)
- [Dual-laser instrument setup \(page 15\)](#)

Requirements for assay setup

Materials required

Before performing setup, ensure you have the following:

- BD FACSComp™ software with BD Calibrite™ 3 beads (Catalog No. 340486) for daily setup
 - BD Calibrite APC beads (Catalog No. 340487) for dual-laser BD FACSCalibur instrument setup
 - BD CellQuest or BD CellQuest Pro software for running the Cytometer Setup Beads
 - Cytometer Setup Beads for setting up the instrument for the assay (included in the BD CBA kit)
 - FITC and PE Positive Control Detectors for setting the initial compensation (included in the BD CBA kit)
 - The appropriate setup template. See [Obtaining the templates \(page 8\)](#).
-

Actions required

Start by performing daily instrument setup using BD FACSComp software and BD Calibrite beads. Run the BD Calibrite beads in lyse/no-wash mode.

If you are using a dual-laser BD FACSCalibur cytometer, ensure that the second laser is turned on.

The data will be evaluated in five parameters (FSC, SSC, FL1, FL2, and FL3 for single-laser instruments and FSC, SSC, FL1, FL2, and FL4 for dual-laser instruments). Turn off additional detectors.

More information

For information on using BD FACSComp software with BD Calibrite beads, see the *BD FACSComp Software Reference Manual* and the *BD Calibrite Beads* package insert.

Single-laser instrument setup

Purpose of the beads

Three tubes of setup beads are required—tubes A, B, and C. Tube A allows you to adjust the PMT voltages, while tubes B and C allow you to adjust compensation.

If this is your first time running the Cytometer Setup Beads, review the information in [Requirements for assay setup](#) (page 10).

Preparing the Cytometer Setup Beads**To prepare the Cytometer Setup Beads:**

1. Add 50 μL of Cytometer Setup Beads to three cytometer setup tubes labeled A, B, and C.
2. Add 50 μL of FITC Positive Control Detector to tube B.
3. Add 50 μL of PE Positive Control Detector to tube C.
4. Incubate tubes A, B, and C for 30 minutes at room temperature, protected from light.
5. Add 450 μL of Wash Buffer to tube A and 400 μL of Wash Buffer to tubes B and C.

Adjusting the cytometer before running the beads

Before running the beads, make the following instrument adjustments:

1. Start BD CellQuest or BD CellQuest Pro software and open the appropriate instrument setup template. See [Obtaining the templates \(page 8\)](#).
2. Set the instrument to Acquisition mode.
3. Set FSC and SSC to Log mode.
4. Decrease the SSC PMT voltage by 100 from what BD FACSComp software set.
5. Set the Threshold to FSC at 650.

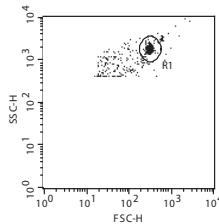
Running the Cytometer Setup Beads

To run the beads:

1. Place tube A on the cytometer. Run the beads in setup mode.

Pause and restart acquisition frequently during the setup procedure to reset the detected values after making adjustments.

2. Adjust gate R1 so that the singlet bead population is located in the gate.

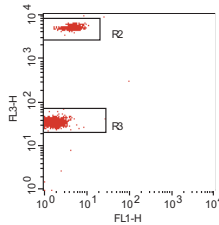


3. Make the following adjustments:

Adjust the FL3 PMT voltage so that the median of the top FL3 bead population's intensity is approximately 5000.

Adjust gate R3 as necessary so that the dim FL3 bead population is located in gate R3. Do not adjust the R2 gate.

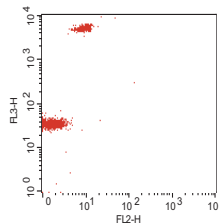
Adjust the FL1 PMT voltage so that the median of FL1 is approximately 2.0 to 2.5.



Bright Beads (R2) Median: 5139.70

FL1 Median: 2.53

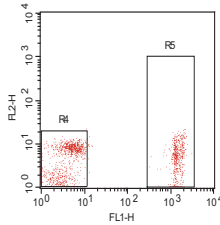
4. Adjust the FL2 PMT voltage so that the median of FL2 is approximately 2.0 to 2.5.



FL2 Median: 2.31

5. Place tube B on the cytometer to adjust the FL2-%FL1 compensation setting.
6. Adjust gate R5 as necessary so that the FL1 bright bead population is located in gate R5.

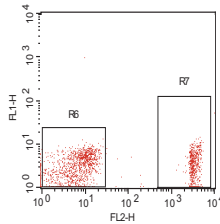
Adjust the FL2-%FL1 compensation setting so that the median of R5 is equal to the median of R4.



R4 Median 2.94 R5 Median 3.75

- Place tube C on the cytometer to adjust the FL1-%FL2 and FL3-%FL2 compensation settings.
- Adjust gate R7 so that the FL2 bright bead population is located in gate R7.

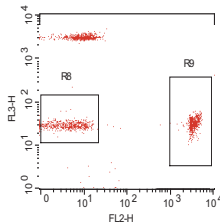
Adjust the FL1-%FL2 compensation setting so that the median of R7 is equal to the median of R6.



R6 Median 2.81 R7 Median 3.08

- Adjust gate R9 so that the FL2 bright bead population is located in gate R9.

Adjust the FL3-%FL2 compensation setting so that the median of R9 is equal to the median of R8.



R8 Median 27.88 R9 Median 28.13

10. Save and print the optimized instrument settings.

Next step

Proceed to [Acquiring samples \(page 20\)](#).

Dual-laser instrument setup

Purpose of the beads

For the dual-laser BD FACSCalibur cytometer, only one tube is required. This tube allows you to adjust the PMT voltages.

Dual-laser setup does not require fluorescence compensation, which simplifies instrument setup and results in fewer problems with samples containing high analyte concentrations.

If this is your first time running the Cytometer Setup Beads, review the information in [Requirements for assay setup \(page 10\)](#).

Preparing the Cytometer Setup Beads

To prepare the Cytometer Setup Beads:

1. Add 50 μL of Cytometer Setup Beads to a tube.
 2. Add 450 μL of Wash Buffer to the tube.
-

Adjusting the cytometer before running the beads

Before running the beads, make the following instrument adjustments:

1. Start BD CellQuest or BD CellQuest Pro software and open the appropriate instrument setup template. See [Obtaining the templates \(page 8\)](#).
2. Set the instrument to Acquisition mode.
3. Set FSC and SSC to Log mode.

4. Decrease the SSC PMT voltage by 100 from what BD FACSComp software set.
5. Set the Threshold to FSC at 650.
6. Set all compensation values to 0.0%.

Any compensation above 0 might adversely affect the performance of the BD CBA assay when using the dual-laser protocol.

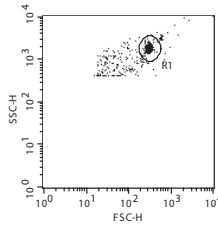
Running the Cytometer Setup Beads

To run the beads:

1. Place the tube on the cytometer. Run the beads in setup mode.

Pause and restart acquisition frequently during the setup procedure to reset the detected values after making adjustments.

2. Adjust gate R1 so that the singlet bead population is located in the gate.

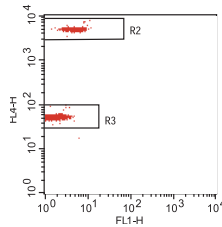


3. Make the following adjustments:

Adjust the FL4 PMT voltage so that the median of the top FL4 bead population's intensity is approximately 5000.

Adjust gate R3 as necessary so that the dim FL4 bead population is located in gate R3. Do not adjust the R2 gate.

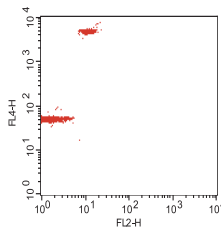
Adjust the FL1 PMT voltage so that the median of FL1 is approximately 2.0 to 2.5.



Bright Beads (R2) Median: 5139.70

FL1 Median: 2.19

- Adjust the FL2 PMT voltage so that the median of FL2 is approximately 2.0 to 2.5.



FL2 Median: 2.21

- Save and print optimized instrument settings.

Next step

Proceed to [Acquiring samples \(page 20\)](#).

3

Acquisition

This section covers the following topics:

- [Acquiring samples \(page 20\)](#)

Acquiring samples

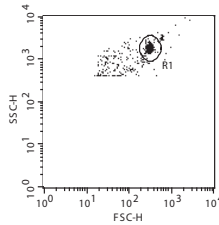
- Before you begin**
- Run the assay setup procedure. See [Cytometer setup \(page 9\)](#) for information.
 - Following the instructions in your BD CBA kit instruction manual to prepare samples.
 - Vortex each sample for 3 to 5 seconds immediately before acquiring on the flow cytometer.
 - Ensure the appropriate acquisition template is available. See [Obtaining the templates \(page 8\)](#).
-

Procedure

To acquire samples using BD CellQuest software:

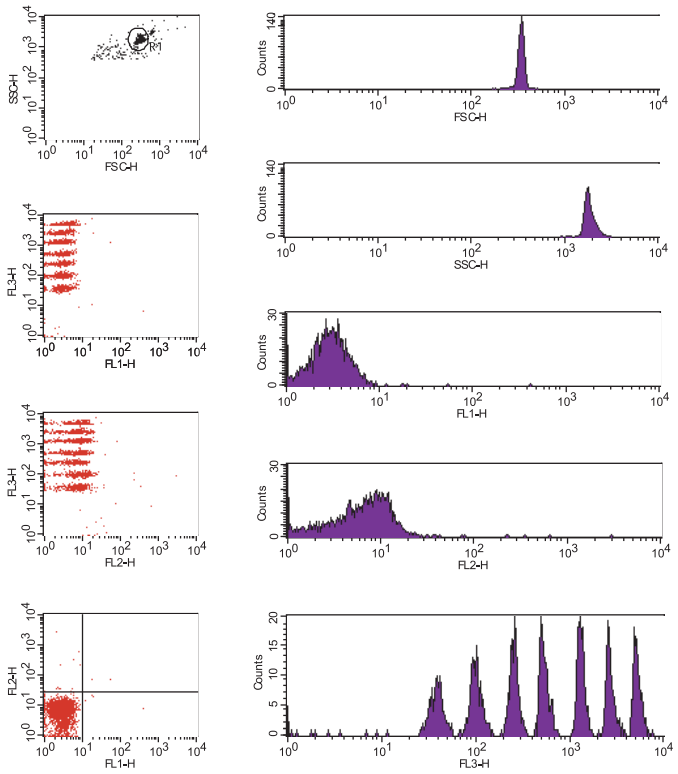
1. Open the appropriate acquisition template.
2. Set acquisition mode and retrieve the optimized instrument settings from [Cytometer setup \(page 9\)](#).
3. In the Acquisition and Storage window, set the resolution to 1024.
4. Set the number of events to be counted to 2100 of R1 gated events. This ensures that the sample file contains approximately 300 events per Capture Bead.
5. Set the number of events to be collected to “all events.” Saving all events collected ensures that no true bead events are lost due to incorrect gating.
6. If you are running the dual-laser protocol, click the **Parameters Saved** button. Ensure that only FSC-H, SSC-H, FL1-H, FL2-H, and FL4-H are selected. It is important that additional detectors be turned off. Click **OK**.

7. Vortex tube 1 (0 standard) for 3 to 5 seconds. Run the tube in setup mode. Using the FSC vs SSC dot plot, place the R1 region gate around the singlet bead population.



8. Specify an alphanumeric file name.
9. Begin sample acquisition with the flow rate set at HIGH. See the following example acquisition template.

Your data might not look exactly like this, but your FL3 (FL4 for dual-laser cytometers) histogram should show similarly well separated peaks.



10. Continue acquiring samples. Vortex each tube for 3 to 5 seconds before acquiring. Run tube 2 (20 pg/mL), followed by tube 3 (40 pg/mL), and so on through tube 10 (Top Standard). Run the unknown samples after the standards.
11. Analyze the data using FCAP Array software. For instructions on analysis, go to bdbiosciences.com/cbasetup and see the *Guide to Analyzing Data from BD CBA Kits Using FCAP Array Software*.

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