Guide for Using BD™ Cytometric Bead Array (CBA) Flex Sets with the BD Accuri™ C6 Flow Cytometer

Technical Bulletin

Contents

Introduction

BD™ Cytometric Bead Array (CBA) flex sets provide a method for capturing a soluble analyte or set of analytes with beads of known size and unique fluorescence, making it possible to detect multiple analytes using flow cytometry. Each capture bead in a BD CBA flex set has been conjugated with a specific antibody. The detection reagent is a mixture of phycoerythrin (PE)–conjugated antibodies, which provides a fluorescent signal proportionate to the amount of bound analyte. When the capture beads and detector reagent are incubated with an unknown sample containing recognized analytes, sandwich complexes (capture bead + analyte + detection reagent) are formed. These complexes can be measured using the BD Accuri™ C6 flow cytometer to identify particles with fluorescence characteristics of both the bead and the detector. Flow cytometry standard (FCS) data files are subsequently analyzed by FCAP Array™ software to generate standard curves and to determine the concentration of unknown samples.

The purpose of this guide is to illustrate the setup procedure on the BD Accuri C6 flow cytometer. Follow the instructions in the BD CBA master buffer kit manual for preparation of samples and use of reagents. Details on analysis can be found in the *FCAP Array™ Software Version 3.0 User's Guide*.

Materials and Methods

Instrumentation

A BD Accuri C6 flow cytometer equipped with the Selectable Laser Module and the following filter configuration.

Detector	Filter	Purpose
FL1	533/30	Not used for BD CBA flex sets
FL2	585/40	PE reporter molecule
FL3	780/60	Bead clustering
FL4	675/25	Bead clustering

Reagents

BD CBA flex sets and a master buffer kit are required to perform the assay. The master buffer kit contains the beads to perform the setup procedure. Visit **bdbiosciences.com/cba** for a complete list of BD CBA flex sets and master buffer kits.

Software

- FCAP Array software v3.0.1 or later (Cat. No. 652099)
- BD Accuri Selectable Laser Module (Cat. No. 653126): Includes the 780/60 filter for FL3

Setup Procedure

Downloading Templates

BD Accuri software templates are available at **bdbiosciences.com/cbasetup** under "Software Templates." Templates have been created for acquisition in either BD Accuri C6 Plus or BD CSampler™ software. Once you have downloaded the files, make sure to select and use the template that is appropriate for the acquisition method being used.

Preparing Setup Beads

Instrument setup beads and wash buffer are provided in the master buffer kit. The F1 bead serves as the unstained population in both setup tubes. Instrument setup bead A1 serves as the positive population in the FL3 channel, while instrument setup bead F9 serves as the positive population in the FL4 channel.

For setup using the tube/tube rack templates:

1. Label two 12 x 75-mm tubes, *F1 + A1* and *F1 + F9*.
2. Add 350 µL of CBA wash buffer to each tube.
3. Add instrument setup beads to the tubes according to the following:
   a. To the F1 + A1 tube, add 25 µL of Instrument Setup Bead F1 and 25 µL of Instrument Setup Bead A1.
   b. To the F1 + F9 tube, add 25 µL of Instrument Setup Bead F1 and 25 µL of Instrument Setup Bead F9.
4. Mix both tubes by vortexing.

For setup using the 96-well plate template:

1. Add 200 µL of CBA wash buffer to wells A1 and A2.
2. Add instrument setup beads to the wells according to the following:
   a. To well A1, add 25 µL of Instrument Setup Bead F1 and 25 µL of Instrument Setup Bead A1.
   b. To well A2, add 25 µL of Instrument Setup Bead F1 and 25 µL of Instrument Setup Bead F9.
3. Mix both wells by pipetting.

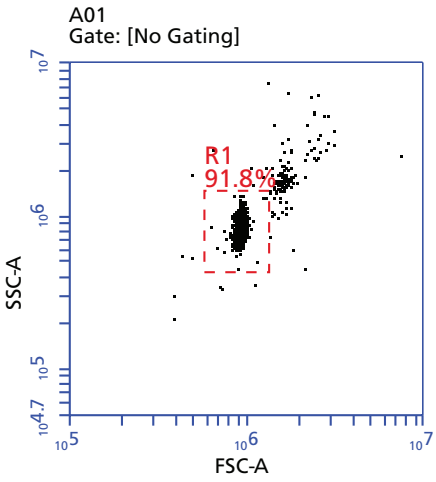


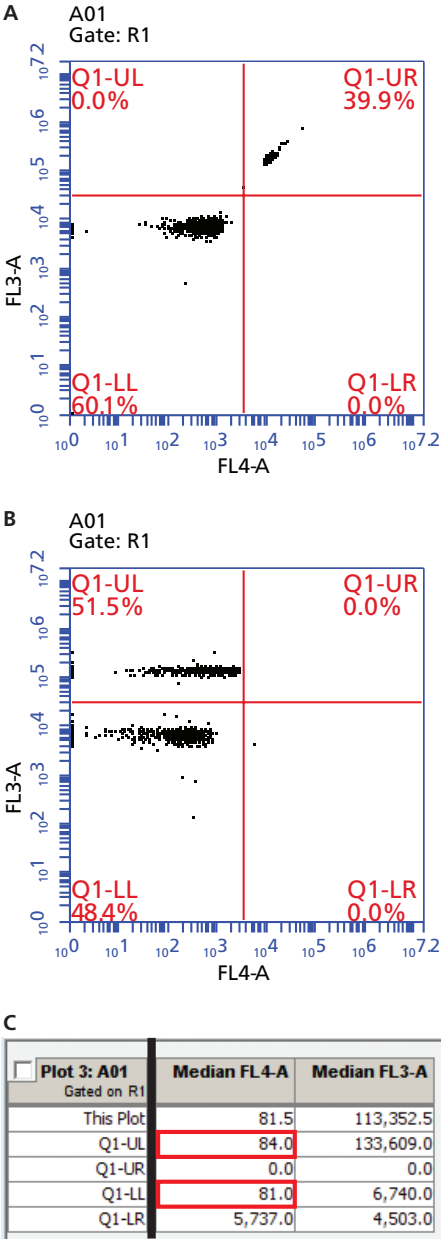
Figure 1.

Setting up the Instrument

This procedure needs to be performed initially to determine the compensation values specific to your instrument. Once these values are established, save the file as a template for the routine acquisition of CBA flex set assays. We recommend performing this procedure once a month to confirm the compensation settings saved in the template. This procedure also needs to be repeated if the instrument has been serviced.

1. Open the template that is appropriate for the acquisition method and plate type that will be used to run the CBA flex sets. The templates contain five plots. Plots 1–3 are used for instrument setup whereas plots 4–5 are optimized for viewing while assay samples are being collected. For instrument setup using the BD CSampler™ accessory, we recommend collecting setup beads in manual collection mode.
2. Select the *2 blue, 2 red* instrument configuration and ensure that the correct filters are installed (see *Materials and Methods*).

   Note: The Selectable Laser Module is required for acquisition of CBA flex sets. If you do not see the option to select the instrument configuration under the well grid, the Selectable Laser Module is not installed.
3. Confirm that the run settings are set to *Run with Limits*, collecting 2,000 events in the R1 gate.
4. Confirm that the flow rate is set to *Medium*.
5. Acquire data for the F1 + A1 tube or well by clicking **Run**. Once acquisition starts, make sure that the R1 gate is capturing the singlet bead population as shown in Figure 1. Move the R1 gate and adjust the plot zoom as necessary.
6. Confirm that the thresholds exclude debris and do not exclude bead events. The default values in the templates are FSC-H and SSC-H, both at 500,000.



C

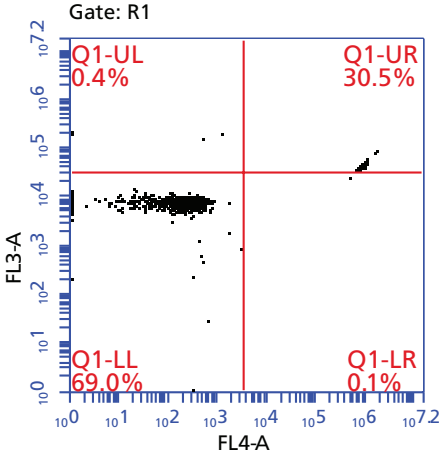
Plot 3: A01 Gated on R1	Median FL4-A	Median FL3-A
This Plot	81.5	113,352.5
Q1-UL	84.0	133,609.0
Q1-UR	0.0	0.0
Q1-LL	81.0	6,740.0
Q1-LR	5,737.0	4,503.0

Figure 2.
A. Uncompensated F1 + A1 data.
B. Compensated F1 + A1 data.
C. Similar FL4 median fluorescence intensities for bead F1 (Q1-LL) and bead A1 (Q1-UL) indicate appropriate compensation.

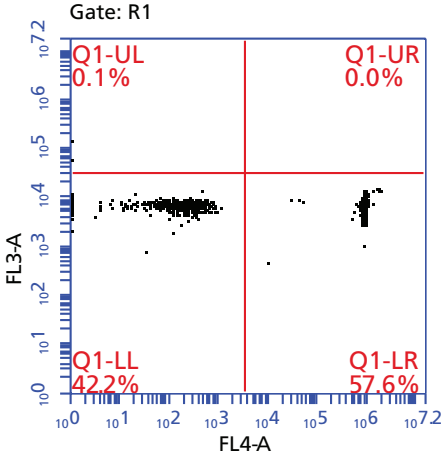
7. Once acquisition has completed, click the **Set Color Compensation** button to set color compensation. A window opens and displays compensation settings for each detector.
8. To correct FL4 by subtracting out a percentage of FL3, click the **FL3** button to enter a compensation value. To avoid overcompensation, we suggest starting with a low compensation value (3–6%). Click the **Preview** button, then check plot 3 and its associated statistics window. As the compensation is increased, the A1 bead population will move from the upper-right quadrant to the upper-left quadrant. Continue increasing the compensation value and clicking the **Preview** button until the median FL4-A value for the upper-left quadrant is similar the median FL4-A value for the lower-left quadrant (Figure 2). The final value is typically <15%. It is better to undercompensate for this step than it is to overcompensate.

   *Note: If median values are not displayed, select **Display > Show Median Statistics**.*
9. Once a compensation value has been determined, select **Apply to: All Samples**, then click **Apply and Close**.

A



B



C

Plot 3: A02 Gated on R1	Median FL4-A	Median FL3-A
This Plot	948,473.5	6,733.0
Q1-UL	0.0	94,319.5
Q1-UR	0.0	0.0
Q1-LL	88.0	6,728.0
Q1-LR	1,027,017.0	6,737.0

Figure 3.
A. Uncompensated F1 + F9 data.
B. Compensated F1 + F9 data.
C. Similar FL3 median fluorescence intensities for bead F1 (Q1-LL) and bead F9 (Q1-LR) indicate appropriate compensation.

10. Select the F1 + F9 tube or well from the well grid and run this sample using the same acquisition settings as the previous tube.
11. Once acquisition has completed, click the **Set Color Compensation** button to set color compensation. A window opens and displays the compensation settings for each detector.
12. To correct FL3 by subtracting a percentage of FL4, click the **FL4** button to enter a compensation value. To avoid overcompensation, we suggest starting with a low compensation value (1–2%). While watching the bead populations in plot 3 and its associated statistics window, increase the compensation value until the median FL3-A value for the lower-right quadrant matches the median FL3-A value for the lower-left quadrant (Figure 3). The final value is typically <5%.
13. Once a compensation value has been established, select **Apply to: All Samples**, then click **Apply and Close**.
14. Save the file as a template for the future acquisition of CBA flex set assays.
    a. Select **File > Save template as.**
    b. Give the template a name and a save location that is convenient for routine use.

Using the Saved Template for Daily Acquisition

1. Perform the assay according to the instructions in the BD CBA master buffer kit manual.
2. Open the template that you created in the previous section.
3. Confirm that the *2 blue, 2 red* option is selected.
4. Set the run settings to collect 300 R1 events for each flex set being run in the multiplex. For example, collect 2,100 events for a 7-plex.
5. Confirm that the flow rate is set to *Medium*.
6. Confirm that the thresholds exclude debris and do not exclude bead events. The default values in the templates are FSC-H and SSC-H, both at 500,000. Thresholds may need to be adjusted after acquisition of the first sample.
7. BD CSampler users can run samples using either *Manual Collect* or *Auto Collect*. If running in *Auto Collect*, we also suggest collecting a few events in manual mode to verify that the instrument settings and gates have been set properly before starting an auto run. Verify that the run settings (compensation, run limits, threshold, and 2 blue, 2 red mode) are also applied correctly in the **Auto Collect** tab prior to starting an auto run.
8. After all samples have been collected, export files by selecting **File > Export ALL samples as FCS.**
9. Analyze data files using FCAP Array software.

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