

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

Technical Bulletin

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Introduction

BD™ Cytometric Bead Array (CBA) kits 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 kit has been conjugated with a specific antibody. The detection reagent provided in the kit 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 the 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 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 standard filter configuration.

Detector	Filter	Purpose
FL1	533/30	Not used for BD CBA kits
FL2	585/40	PE reporter molecule
FL3	670 LP	Not used for BD CBA kits
FL4	675/25	Bead clustering

Reagents

A BD CBA kit is required to perform the assay. Visit bdbiosciences.com/cba for a complete list of BD CBA kits.

Software

FCAP Array software v3.0.1 or later (Cat. No. 652099)

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 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.

Acquiring Data

Due to the combined properties of the CBA reagents and the optical configuration of the BD Accuri C6, the setup beads that are provided with the BD CBA kits are not used and color compensation is not required. Perform the following steps after incubation of standards and samples as described in the BD CBA kit manual.

1. Open the template that is appropriate for the acquisition method and plate type that will be used to run the BD CBA kit.
2. Confirm that the run settings are set to *Run with Limits*, collecting 2,100 events in the R1 gate.
3. Confirm that the flow rate is set to *Medium*.
4. Select the tube or well that corresponds to the 0 pg/mL standard and click **Run**.
5. Once acquisition is complete, 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. Confirm that the expected number of bead peaks is visible on the FL4 histogram (Figure 1).
6. Run the remaining standards and samples using *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 (eg, run limits and thresholds) are also applied correctly in the **Auto Collect** tab prior to starting an auto run.
7. After all samples have been collected, export files by selecting **File > Export ALL samples as FCS**.
8. Analyze data files using FCAP Array software.

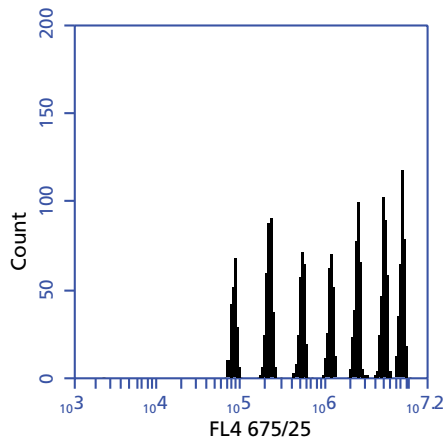


Figure 1. The seven bead populations in a BD CBA Human Th1/Th2/Th17 kit.



BD Biosciences
2350 Qume Drive
San Jose, CA 95131
US Orders: 855.236.2772
Technical Service: 877.232.8995
answers@bd.com
bdbiosciences.com

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23-14178-00