BD™ Cytometric Bead Array (CBA)

Acquisition Using the BD Multiwell™ AutoSampler

Deep-Well Plate Protocol Manual

BD

BD Biosciences

For Research Use Only. Not for use in diagnostic or therapeutic procedures. Purchase does not include or carry any right to resell or transfer this product either as a stand-alone product or as a component of another product. Any use of this product other than the permitted use without the express written authorization of Becton Dickinson and Company is strictly prohibited.

BD, BD Logo and all other trademarks are the property of Becton, Dickinson and Company. ©2003 BD

Table of Contents

Introduction 4

Materials Required, but not Provided. 4

Optional Materials not Provided 4

Required Files 4

Deep-Well Plate Assay Protocol 5

Cytometer Setup, Data Acquisition, and Analysis. 5

- Preparation of Cytometer Setup Beads 5
- Instrument Setup with BD FACSComp™ Software and BD CaliBRITE™ Beads 6
- Instrument Setup with the Cytometer Setup Beads 6
- Preparation of the Acquisition Template 10

Setup of the BD Multiwell™ AutoSampler 11

Introduction

The BD Multiwell™ AutoSampler (MAS) is an automated sample introduction instrument that enables the analysis of samples directly from 96- or 384-well plates using a BD Biosciences flow cytometer. A BD flow cytometer installed with the MAS upgrade and the BD CellQuest™ and Multiwell Plate Manager™ (MPM) Software will provide the operator with an automated sample analysis system. This document describes protocols for performing BD™ Cytometric Bead Array (BD™ CBA) experiments for analysis using a flow cytometer equipped with the MAS.

The protocol described here is for performing BD CBA experiments in Deep-Well 96-well plates. Performing BD CBA experiments in a plate format does not affect the overall performance of the assay.

Materials Required, but not Provided

Deep-Well 96-well plates, 2.0 ml conical non-sterile polypropylene (BD Falcon Cat. No. 353966, or equivalent).

Plate Sealers, pressure sensitive, fit 96-well plates, non-sterile (Costar, Cat. No. 3095).

Vortex, Vortex Genie 2 (Fisher Cat. No. 12-812, or equivalent).

Vortex 96-well plate adapter (Fisher Cat. No. 12-812C, or equivalent).

Swing bucket centrifuge (Sorvall RT6000B [**www.kendro.com**], or equivalent).

96-well plate centrifuge adapter (Kendro/Sorvall Part. No. 11093 [**www.kendro.com**], or equivalent).

Aspiration manifold, 8- or 12-channel, 30-mm depth, V&P Scientific (**www.vp-scientific.com**), 8-channel (Cat. No. VP-180) or 12-channel (Cat. No. VP-187A) (specify depth when ordering).

Aspiration apparatus.

Optional Materials not Provided

Multichannel pipette, 8- or 12-channel, Eppendorf 1200, or equivalent.

Repeating pipette, Eppendorf Combi tip, or equivalent.

Required Files

It is essential that the operator generate the following critical files before acquisition of BD CBA samples using the MAS. These files include an instrument settings file and a BD CellQuest acquisition template. The creation of these files and settings is described below.

Unless otherwise specified, all products are for Research Use Only. Not for use in diagnostic or therapeutic procedures. Not for resale.

Deep-Well Plate Assay Protocol

Performing a BD CBA experiment in a deep well 96-well plate is very similar to the protocol described in the BD CBA Kit manual. Follow all information in the manual for the BD CBA kit being assayed. The following plate-specific instructions are the only modifications to the BD CBA assay protocol described in the BD CBA Kit manual.

1. Add reagents to the Deep-Well plate in accordance with the BD CBA Kit manual. (Add beads, detection reagents, and sample in the same volumes and order as described in the BD CBA Kit manual.)
2. Cover the plate using a plate sealer.
3. Incubate the plate as described in the Assay Protocol in the BD CBA Kit manual.
4. To wash, add 1,000 µl cold Wash Buffer per well, and mix the covered plate using vortex and plate adapter.
5. Centrifuge plate in swing bucket centrifuge equipped with a 96-well plate adapter for 7 minutes at 200 × *g*.
6. Aspirate supernatant using 8- or 12-channel manifold.
7. Resuspend beads in 200 µl Wash Buffer per well and mix plate using vortex and plate adapter. The plate is now ready for acquisition.
8. Perform Instrument Setup (see ***Cytometer Setup, Data Acquisition, and Analysis***) and begin sample acquisition.

Cytometer Setup, Data Acquisition, and Analysis

The cytometer setup information in this section is for the BD FACScan™ and BD FACSCalibur™ System flow cytometers. The BD FACSComp™ Software is useful for setting up the flow cytometer. BD CellQuest Software is required for analyzing samples and formatting data for subsequent analysis using the BD CBA Software.

Preparation of 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 and protect from direct exposure to light.
5. Add 450 µl of Wash Buffer to tube A and 400 µl of Wash Buffer to tubes B and C.
6. Proceed to Instrument Setup with BD FACSComp™ Software and BD CaliBRITE™ Beads.

Unless otherwise specified, all products are for Research Use Only.
Not for use in diagnostic or therapeutic procedures. Not for resale.

Instrument Setup with BD FACSComp™ Software and BD CaliBRITE™ Beads:

1. Perform instrument start up.
2. Perform flow check.
3. Prepare tubes of BD CaliBRITE beads and open the BD FACSComp Software.
4. Launch the BD FACSComp Software.
5. Run the BD FACSComp Software in Lyse/No Wash mode.
6. Proceed to **Instrument Setup with the Cytometer Setup Beads**.

   Note: For detailed information on using BD FACSComp with BD CaliBRITE beads to set up the flow cytometer, refer to the *BD FACSComp Software User's Guide* and the *BD CaliBRITE Beads* package insert. Version 4.2 contains a BD CBA preference setting to automatically save a BD CBA calibration file at the successful completion of any Lyse/No Wash assay. The BD CBA calibration file provides the optimization for FSC, SSC, and threshold settings as described in Instrument Setup with the Cytometer Setup Beads, Steps 3 - 5. Optimization of the fluorescence parameter settings is still required (ie, PMT and compensation settings, see *Instrument Setup with the Cytometer Setup Beads*, Step 6).

Instrument Setup with the Cytometer Setup Beads:

1. Launch BD CellQuest Software and open the BD CBA Instrument Setup template.

   Note: The BD CBA Instrument Setup template can be found on the BD CBA Software or FACStation CD (prior to version 3.5) for Macintosh computers in the BD CBA folder. Following installation on Macintosh computers using BD CBA Software Version 1.0, the template can be found in the BD Applications /BD CBA folder/ Samples Files/Mouse Isotyping Files/Instrument Setup folder. For BD CBA Software Version 1.1 or higher, the template can be found in the BD Applications/BD CBA folder. The template is not installed from the CD on PC-compatible computers. This file and instrument setup templates for dual-laser and other flow cytometers may also be downloaded via the internet from: **http://www.bdbiosciences.com/pharmingen/CBA/downloads.shtml**

2. Set the instrument to Acquisition mode.

   Note: The BD CBA Software will evaluate data in five parameters (FSC, SSC, FL1, FL2, and FL3). Turn off additional detectors.

3. Set SSC (side light scatter) and FSC (forward light scatter) to Log mode.
4. Decrease the SSC PMT voltage by 100 from what FACSComp set.
5. Set the Threshold to FSC at 650.

*Unless otherwise specified, all products are for Research Use Only.
Not for use in diagnostic or therapeutic procedures. Not for resale.*

6. In Setup mode, run Cytometer Setup Beads tube A. Follow the setup instructions on the following pages.

   Note: Pause and start acquisition frequently during the instrument setup procedure in order to reset detected values after settings adjustments.

Adjust gate R1 so that the singlet bead population is located in gate R1 (***Figure 1***).

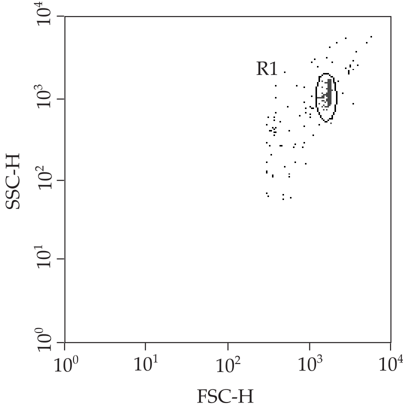
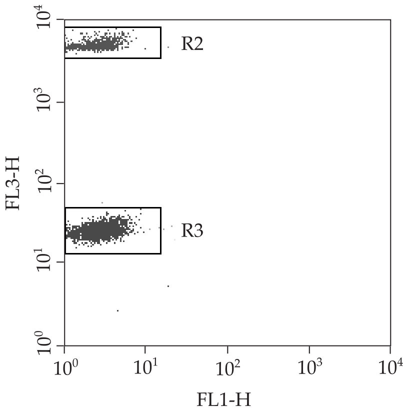


Figure 1

Adjust the FL3 PMT so that the median of the top FL3 bead population's intensity is around 5000 (***Figure 2***). Adjust gate R3 as necessary so that the dim FL3 bead population is located in gate R3 (***Figure 2***). Do not adjust the R2 gate.



Bright Beads (R2)	Median: 5139.70
FL1 (Median)	Median: 2.53

Figure 2

Adjust the FL1 PMT so that the median of FL1 is approximately 2.0 - 2.5 (***Figure 2***).

Unless otherwise specified, all products are for Research Use Only.
Not for use in diagnostic or therapeutic procedures. Not for resale.

Adjust the FL2 PMT so that the median of FL2 is approximately 2.0 - 2.5 (***Figure 3***).

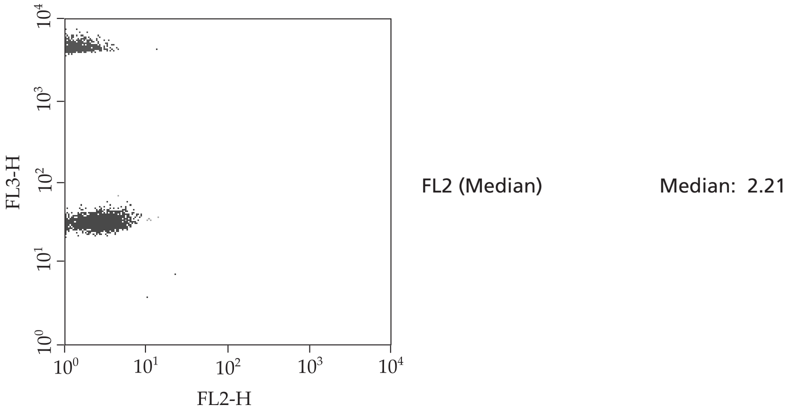


Figure 3

Run Cytometer Setup Beads tube B to adjust the compensation settings for FL2 - %FL1.

Adjust gate R5 as necessary so that the FL1 bright bead population is located in gate R5 (***Figure 4***). Using the FL2 - %FL1 control, adjust the median of R5 to equal the median of R4 (***Figure 4***).

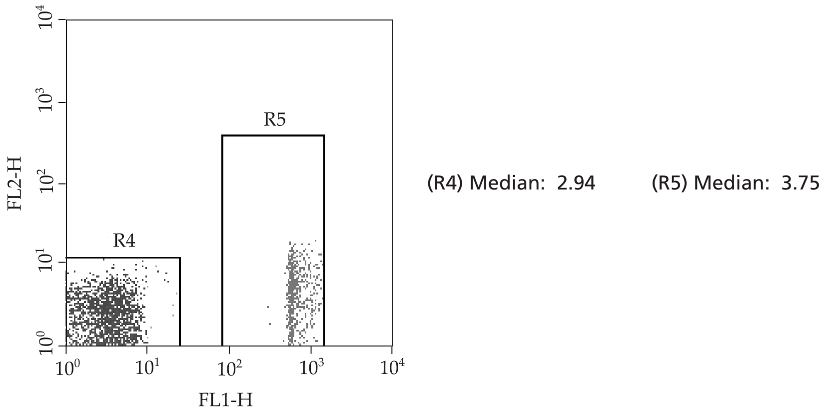


Figure 4

Run Cytometer Setup Beads tube C to adjust the compensation settings for FL1 - %FL2 and FL3 - %FL2.

Unless otherwise specified, all products are for Research Use Only.
Not for use in diagnostic or therapeutic procedures. Not for resale.

Adjust gate R7 so that the FL2 bright bead population is located in gate R7 (***Figure 5***). Using the FL1 - %FL2 control, adjust the median of R7 to equal the median of R6 (***Figure 5***).

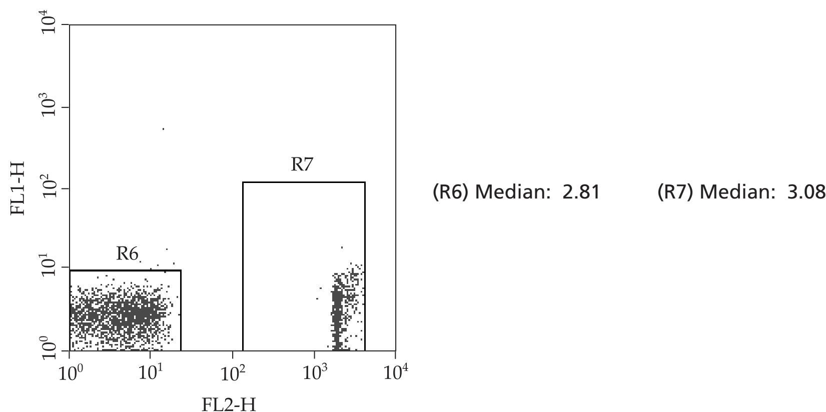


Figure 5

Adjust gate R9 so that the FL2 bright bead population is located in gate R9 (***Figure 6***). Using the FL3 - %FL2 control, adjust the median of R9 to equal the median of R8 (***Figure 6***).

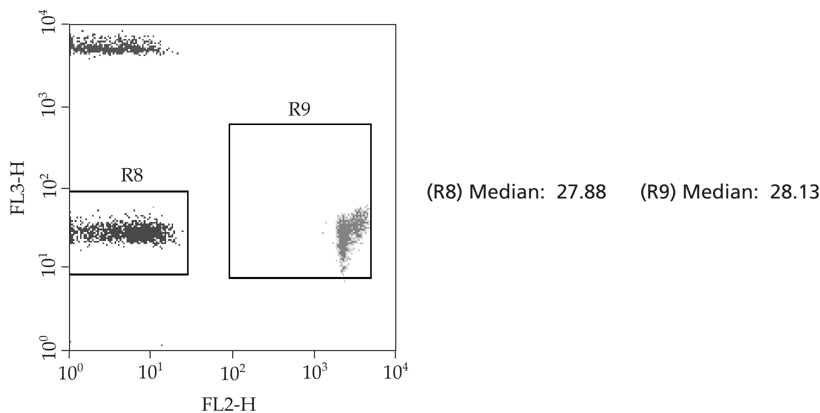


Figure 6

Set the FL2 - %FL3 to 0.1 if necessary. Print the optimized instrument settings; save the settings in an instrument settings file. Note the location of the saved file for future use.

Unless otherwise specified, all products are for Research Use Only.
Not for use in diagnostic or therapeutic procedures. Not for resale.

Preparation of the Acquisition Template:

1. Open the Acquisition template on the BD CBA Software.

   Note: Following installation of the BD CBA Software, the Acquisition template can be found in the BD Applications/BD CBA folder/Samples Files/Mouse Isotyping Files/Instrument Setup folder and is labeled "Isotype Kit Acquire Template". Alternatively, the Acquisition template may be downloaded via the internet from: **www.bdbiosciences.com/pharmingen/CBA/downloads.shtml**

2. Set Acquisition mode and retrieve the optimized instrument settings from Instrument Setup with the Cytometer Setup Beads.
3. In the Acquisition and Storage window, set the resolution to 1024. Set collection criteria to Event Count or Time.
4. Set the number of events to be counted as described in the specific BD CBA Kit manual (R1 gated events). This will ensure 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 will ensure that no true bead events are lost due to incorrect gating.
6. Set the stop time to 59 seconds.
7. Prepare a tube containing mixed capture beads from the BD CBA Kit, and dilute with 400 µl of Wash Buffer.
8. In Setup mode, run the bead tube, and using the FSC vs. SSC dot plot, place the R1 region gate around the singlet bead population (see ***Figure 1***).
9. Save the Acquisition template and note the location of the saved file for future use.

Unless otherwise specified, all products are for Research Use Only. Not for use in diagnostic or therapeutic procedures. Not for resale.

Setup of the BD Multiwell™ AutoSampler

It is essential that the user be familiar with the MPM Software and the use of the MAS. It is recommended that the user read pertinent information in the *MAS Software User's Guide* and the *MPM Software User's Guide*. Use the following BD CBA-specific settings for running samples with the MAS.

1. Attach the MAS Cytometer Interface Unit (CIU) to the Sample Injector Port of the flow cytometer as described in the *MAS Software User's Guide*. Remove the Droplet Containment Module (DCM) first, if installed.
2. Open the MPM Software, choose Acquisition for the MPM mode, and login on the MPM:Login screen.
3. Highlight all appropriate wells used in the assay on the MPM plate template.
4. Select the Instrument Settings File and Acquisition Template prepared and saved in Cytometer Setup, Data Acquisition, and Analysis.
5. Set the plate definition. The definition for the Deep-Well 96-well plate is 353966.
6. Set Background to None.
7. Set the sample and mix volumes to 100 µl and set the number of mixes to two. Set the number of washes to 1.
8. Set the data storage folder.
9. Set the data prefixes according to user preference in accordance with the *MPM Software User's Guide*.
10. Under AutoSampler, choose Prime System twice.
11. To keep these settings for subsequent runs, select File, Save, and Protocol. Assign an appropriate name for the protocol and click Save. For future runs, select File, Open, Protocol, and the name of the protocol to recall the settings for the BD CBA assay.
12. Begin sample acquisition by selecting Acquisition from the menu, then Acquire. When the MPM control window appears, select Acquire.

    Note: Refer to the BD CBA Kit manual and the *BD CBA Software User's Guide* (PN 341775) for information regarding analysis of sample files using the BD CBA Software.

Notes

Notes

Notes

United States
877.232.8995

Canada
888.259.0187

Europe
32.53.720.211

Japan
0120.8555.90

Asia/Pacific
65.6861.0633

Latin America/Caribbean
55.11.5185.9995

BD Biosciences

BD Biosciences Pharmingen
10975 Torreyana Road
San Diego, CA 92121
Customer/Technical Service
Tel 877.232.8995 (US)
Fax 858.812.8888
www.bdbiosciences.com

02-8100055-23A