

Guide to Using BD FACSDiva
Software with
BD Cytometric Bead Array
(CBA) Kits



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History

Revision	Date	Change made
645070 Rev A	7/09	New document

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Who should read this guide

About this topic	To help you determine whether you should read this guide, this topic explains the purpose of the guide and lists the products to which it applies.
Purpose of this guide	The purpose of this guide is to provide setup and acquisition instructions for researchers who are planning to use a BD FACST [™] digital flow cytometer to acquire data obtained with a BD [™] Cytometric Bead Array (CBA) kit.
Flow cytometers	<p>The procedures in this guide are for use with one of the following digital flow cytometers:</p> <ul style="list-style-type: none">• BD[™] LSR II flow cytometer with 488-nm and 635-nm lasers• BD FACSCanto[™] II flow cytometer with 488-nm and 633 nm lasers• BD FACSAria[™] II flow cytometer with 488-nm and 633-nm lasers
Software	This guide assumes that you are using BD FACSDiva [™] software version 6.0 or later.
BD CBA kits	The procedures in this guide were 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.

Kit name	Catalog number
Human Anaphylatoxin Kit	552363
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

Related topics

- [How this guide relates to other BD CBA guides \(page 2\)](#)

How this guide relates to other BD CBA guides

About this topic To help you determine when to use this guide and when to use related guides, this topic explains where to find instructions for each of the basic stages of the BD CBA assay workflow.

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 one of the

BD FACS digital flow cytometers listed in [Who should read this guide \(page 1\)](#).

For information about...	See...
1. Preparing standards and samples	The instruction manual that came with your kit
2. Setting up using BD FACSDiva software	This guide
3. Acquiring data	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/cbakits

Cytometer instructions in your kit manual

If your kit manual describes setup and acquisition procedures for a flow cytometer system that is different from the one you are using, skip those instructions and follow the procedures in this guide instead.

Related topics

- [Who should read this guide \(page 1\)](#)
 - [Obtaining the template \(page 4\)](#)
-

Obtaining the template

About this topic BD has created a BD FACSDiva template for you to use with BD CBA kits. This topic explains how to obtain the template from the BD Biosciences website.

About the template The *Digital Instrument CBA Template* is a file that you can import into BD FACSDiva software to save time. It contains two predefined worksheets, one for setup and one for acquisition.

When to perform this procedure You only need to obtain the template once, after which you can use it as many times as you need to.

Procedure To obtain the template:

1. Navigate to bdbiosciences.com/cbatemplates.
2. Download the *Digital Instrument CBA Template*.
3. After the download is complete, unzip the file.
4. Without changing the file name, save the file in D:\BDEExport\Templates\Experiment.

Do not change the name of the Templates folder.

Next step Proceed to [Setting up using BD FACSDiva software \(page 5\)](#).

Related topics

- [Who should read this guide \(page 1\)](#)

Setting up using BD FACSDiva software

About this topic This topic describes how to adjust photomultiplier tube (PMT) settings in preparation for running your BD CBA assay using BD FACSDiva software.

When to perform this procedure You must perform this setup procedure if one or more of the following is true:

- You are using a new type of BD CBA kit for the first time
 - It has been more than a month since you last performed this procedure
 - Your flow cytometer has been serviced since you last performed this procedure
-

When to skip this procedure You can skip this procedure if none of the above statements is true and you have access to a saved experiment file containing assay-specific settings (you will save this file in the final step of this procedure).

Before you begin If necessary, complete the steps described in [Obtaining the template \(page 4\)](#).

Ensure that you have the following materials:

- One 12 x 75-mm sample acquisition tube for a flow cytometer (eg, BD Falcon™ Catalog No. 352008)
- At least 100 µL of cytometer setup beads (vial D in your BD CBA kit) and 400 µL of wash buffer (bottle F).

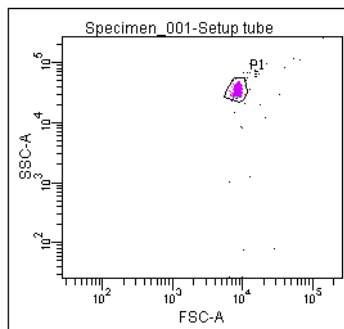
Note: The positive control detectors in vials E1 and E2 are not used in this procedure.

Preparing for setup**To prepare for setup:**

1. Add 100 μL of cytometer setup beads (vial D) to a 12 x 75-mm sample acquisition tube.
2. Add 400 μL of wash buffer and vortex the tube gently to mix the contents.
3. In BD FACSDiva software, select **Experiment > New Experiment** and open the *Digital Instrument CBA Template*.
4. In the **Global Worksheet**, click the **CBA Instrument Setup** tab.
5. Set the **Current Tube Pointer** to indicate the tube with the setup beads.

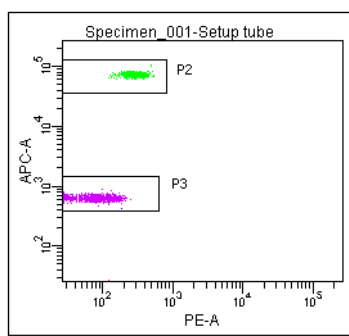
Performing setup**To perform setup:**

1. Load the tube of prepared beads onto the cytometer and begin acquiring.
2. Adjust the FSC and SSC voltages until the singlet bead population fits within P1.



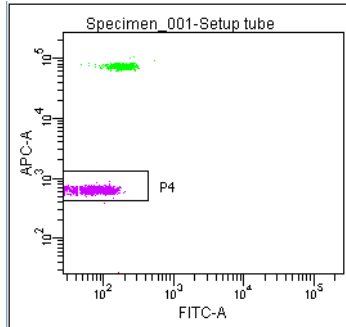
3. Adjust the APC voltage until the mean of P2 is approximately 70,000.

- Adjust the PE voltage until the mean of P3 is approximately 75.



Experiment Name: Digital Instrument CBA Setup		
Specimen Name: Specimen_001		
Tube Name: Setup tube		
Record Date: Jul 28, 2008 1:49:56 PM		
Population	PE-A Mean	APC-A Mean
■ P2	290	73,793
■ P3	76	631

5. Adjust the FITC voltage until the mean of P4 is approximately 75.



Experiment Name: Digital Instrument CBA Setup	
Specimen Name: Specimen_001	
Tube Name: Setup tube	
Record Date: Jul 28, 2008 1:49:56 PM	
Population	FITC-A
■ P4	Mean
	73

6. Stop acquiring and unload the tube.
7. Save your assay-specific settings for future use:
 - a. In the **Browser**, right-click **Cytometer Settings** and select **Save to Catalog**.
 - b. Name the file, then click **OK**.

Next step

Proceed to [Acquiring data \(page 9\)](#).

Related topics

- [Who should read this guide \(page 1\)](#)
 - [Obtaining the template \(page 4\)](#)
-

Acquiring data

About this topic This topic describes how to acquire data from a BD CBA assay using a BD FACS digital flow cytometer.

Before you begin Prepare your CBA standards and samples following the instructions in your BD CBA kit manual.

Perform the procedure described in [Setting up using BD FACSDiva software \(page 5\)](#).

If you skipped the setup procedure, start BD FACSDiva software and import the experiment file that contains your assay-specific settings.

Procedure

To acquire data:

1. In the **Global Worksheet**, click the **CBA Acquisition** tab.
2. Add tubes to the BD FACSDiva experiment and label them appropriately for each of your standards (in order of increasing concentration) and each of your samples.
3. Set the **Current Tube Pointer** to indicate the 0 pg/mL standard tube.
4. Load the 0 pg/mL standard tube on the cytometer and begin acquiring.
5. If necessary, adjust P1 to include only the singlet beads.
6. Record data from the 0 pg/mL tube.
7. Record data from each standard tube in order of increasing concentration.
8. Record data from each of your sample tubes.

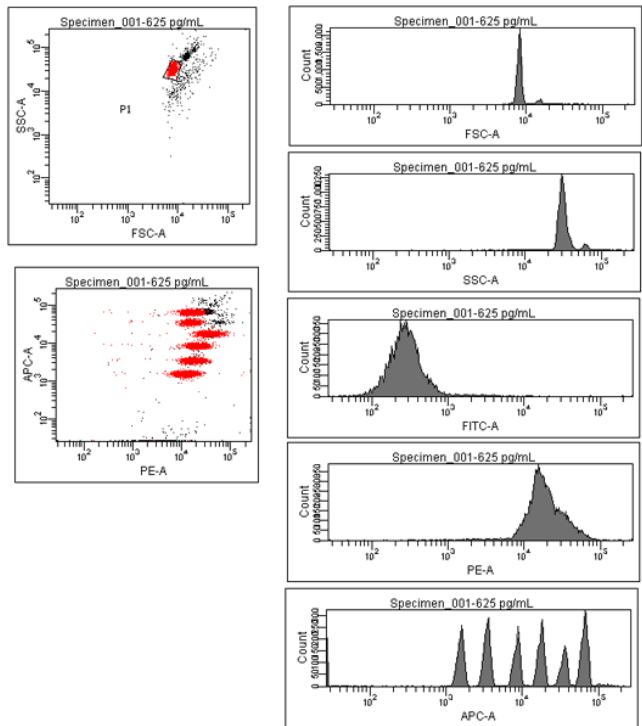
Next step

If necessary, proceed to [Exporting data for analysis](#) (page 11).

Example data

This illustration shows an example of data obtained using a BD CBA kit.

Your data might not look exactly like this, but your APC-A histogram should show similarly well separated peaks.



Related topics

- [Setting up using BD FACSDiva software \(page 5\)](#)
-

Exporting data for analysis

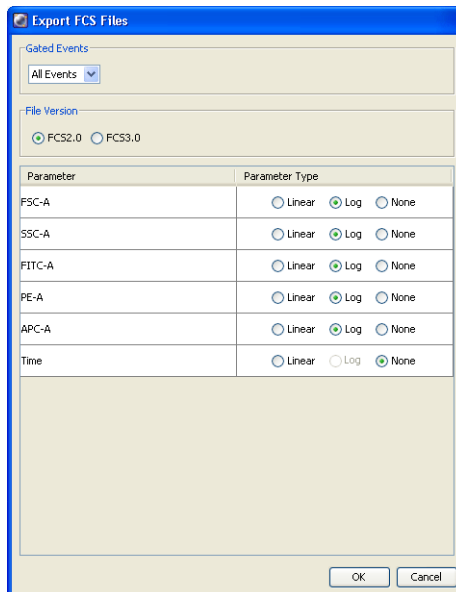
About this topic This topic explains how to export data for analysis. This procedure generates an individual FCS file for each tube.

Procedure

To export data for analysis:

1. In BD FACSDiva software, select the experiment, specimen, or tube(s) you want to export. You can select multiple items for simultaneous export.
2. Select **File > Export > FCS files**.

The **Export FCS Files** dialog appears.



3. Select **All Events** from the **Gated Events** menu.
4. Select the appropriate file version.

If you will be analyzing data in FCAP Array software, you must select **FCS 2.0**.

5. Select **Log** to export the following parameters:

- FSC-A
- SSC-A
- FITC-A
- PE-A
- APC-A

6. Select **None** for all other parameters.

7. Click **OK**.

8. In the **Save Export** dialog, specify the file storage location.

You can click **Details** to view the directory path and file name for each exported tube. The file names are the tube names and cannot be changed.

9. Click **Save** to export the files.

More information

For data analysis instructions, see the *Guide to Analyzing Data from BD Cytometric Bead Array (CBA) Kits Using FCAP Array Software*.

This guide can be found at bdbiosciences.com/cbakits.

Related topics

- [How this guide relates to other BD CBA guides \(page 2\)](#)
-

United States

877.232.8995

Canada

888.259.0187

Europe

32.2.400.98.95

Japan

0120.8555.90

Asia/Pacific

65.6861.0633

Latin America/Caribbean

55.11.5185.9995



BD Biosciences

2350 Qume Drive

San Jose, CA 95131-1807

Toll free: 877.232.8995 (US)

Tel: 408.432.9475

Fax: 408.954.2347

bdbiosciences.com