

Guide to Using  
BD FACSuite™ Software  
with BD™ Cytometric Bead  
Array (CBA) Products



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## History

Revision	Date	Change made
23-12943-00 Rev. 01	8/2011	New document
23-12943-01 Rev. 01	2/2012	Updated assay setup instructions.

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# 1

## Getting started

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This chapter covers the following topics:

- [BD CBA overview \(page 6\)](#)
- [How this guide relates to other BD user's guides \(page 7\)](#)
- [Workflow \(page 8\)](#)
- [Obtaining setup experiments \(page 9\)](#)
- [Importing setup experiments \(page 11\)](#)

## BD CBA overview

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**Introduction** The purpose of this guide is to provide assay setup instructions for researchers who use BD FACSuite™ software to acquire data obtained with a BD™ Cytometric Bead Array (CBA) kit or flex set.

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**Flow cytometers** The procedures in this guide are for use with the following digital flow cytometer:

- BD FACSVerser™ flow cytometer with at least two lasers, 488-nm and 640-nm laser

**Note:** If the BD FACST™ Universal Loader is being used with filter plates, you must use Millipore Catalog No. MABVN12. This plate is different than the one recommended in the BD CBA reagent manuals.

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**Software** This guide assumes that you are using the following:

- BD FACSuite software version 1.0 or later
- FCAP Array™ software version 3.0 or later

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**BD CBA products** The procedures in this guide were designed for the following:

- BD CBA kits with single-color capture beads and PE or PE and FITC detection reagents
- BD CBA flex sets with dual-color capture beads and PE detection reagent

**Parameters for analysis**

The procedures in this guide require the following parameters for analysis:

- BD CBA kits use CBA Red and PE
- BD CBA flex sets use CBA Red, CBA NIR, and PE

## How this guide relates to other BD user's guides

**Introduction**

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 five basic stages. The following table describes where to find instructions for each stage.

For information about...	See...
1. Preparing standards and samples	The instruction manual that came with your kit or flex set
2. Setting up your CBA assay in BD FACSuite software	This guide
3. Using keywords to establish an interface between BD FACSuite software and FCAP Array software	This guide
4. Acquiring data	<i>BD FACSVersé System Reference</i>
5. Analyzing data	<i>FCAP Array Software Version 3.0 User's Guide</i> , found at: <a href="http://bdbiosciences.com/cbasetup">bdbiosciences.com/cbasetup</a>

## Workflow

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**Introduction** This topic explains the setup workflow, both initially and routinely.

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**BD CBA initial setup workflow** The following table shows the typical stages of the initial BD CBA setup workflow.

Stage	Description
1	<a href="#">Obtaining setup experiments (page 9)</a>
2	<a href="#">Assigning CBA fluorochromes to detectors (page 10)</a>
3	<a href="#">Importing setup experiments (page 11)</a>
4	Running performance QC
5	Assay & Tube Settings setup
6	<a href="#">Running assay setup for BD CBA kits (page 16)</a> or <a href="#">Running assay setup for BD CBA flex sets (page 22)</a>
7	<a href="#">Setting FCS export options (page 34)</a>
8	<a href="#">Adding standards, samples, and controls to a worklist (page 35)</a>
9	<a href="#">Assigning values to BD CBA keywords (page 40)</a>



**BD CBA routine setup workflow**

The following table shows the stages of the routine setup for subsequent days once the initial setup has been completed.

Stage	Description
1	Running performance QC
2	Assay & Tube Settings setup
3	<a href="#">Adding standards, samples, and controls to a worklist (page 35)</a>
4	<a href="#">Assigning values to BD CBA keywords (page 40)</a>

**Obtaining setup experiments****Introduction**

This topic explains how to obtain the setup experiment files from the BD Biosciences website.

**About the setup experiments**

We have created BD FACSuite setup experiments for you to use with your BD CBA products. The BD CBA setup experiments are files that you import into BD FACSuite software to perform assay setup.

There are two sets of experiments.

- BD CBA kits. One experiment for standards and one experiment for samples.
- BD CBA flex sets. One experiment for standards and one experiment for samples.

Download the experiments that are appropriate for the reagents that you are using.

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

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**Procedure** To download the setup experiments:

1. Navigate to [bdbiosciences.com/cbasetup](http://bdbiosciences.com/cbasetup).
2. Download either the BD CBA kit experiments or the BD CBA flex set experiments (or both if you use a kit and flex set).
3. After the download is complete, unzip the files.
4. Without changing the file names, save the files in the following folder:  
C:\BD Import\BDFSExperiment

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**Next step** Proceed to [Assigning CBA fluorochromes to detectors \(page 10\)](#).

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## Assigning CBA fluorochromes to detectors

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**Introduction** This topic describes how to assign the CBA fluorochromes to their respective detectors in BD FACSuite software.

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**Procedure** To assign the CBA fluorochromes to detectors:

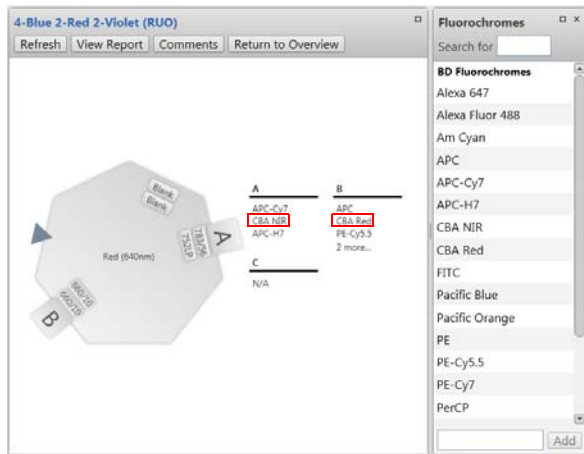
1. In the **Setup and QC** workspace, click the **Configurations** tab.

2. Select the CBA Red fluorochrome, then drag and drop it onto the list of fluorochromes for detector B of the red laser.

Set it as the secondary fluorochrome.

3. Select the CBA NIR fluorochrome, then drag and drop it onto the list of fluorochromes for detector A of the red laser.

Set it as the secondary fluorochrome.



## Next step

Proceed to [Importing setup experiments \(page 11\)](#).

## Importing setup experiments

### Introduction

This topic describes how to import the BD CBA setup experiments into BD FACSuite software.

The BD CBA setup experiments contain tube settings. Once imported, the tube settings are available in your BD FACSuite library.

Also included in the setup experiments are plots, statistics tables, and CBA keywords.

**Note:** If you have previously imported these experiments and not renamed them in BD FACSuite software, the new experiments will import with numbers appended to the end of the names, for example BD CBA Kit Standards 001.

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## Procedure

To import the setup experiments:

1. In the **Experiment** workspace, click the **Manage Experiments** tab.
2. In the **Experiments Browser**, click an experiment folder.
3. Select **File > Import Experiments**.

The **Import Experiments** dialog opens.

4. Navigate to C:\BD Import\BDFSExperiment.
5. Select the standards experiment that you downloaded and click **Open**.

The standards experiment becomes the active experiment. Close the experiment.

6. Repeat steps 1 to 4.
7. Select the samples experiment that you downloaded and click **Open**.

The samples experiment becomes the active experiment. Close the experiment.

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**Next step**

If you are using a BD CBA kit, then proceed to [Running assay setup for BD CBA kits \(page 16\)](#).

If you are using a BD CBA flex set, then proceed to [Running assay setup for BD CBA flex sets \(page 22\)](#).

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# 2

## Assay setup

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The chapter covers the following topics:

- [Running assay setup for BD CBA kits \(page 16\)](#)
- [Running assay setup for BD CBA flex sets \(page 22\)](#)

## Running assay setup for BD CBA kits

**Introduction** This topic describes how to confirm the BD CBA kit tube settings that were imported as part of the setup experiments and to create assays from the standards and samples setup experiments.

**Before you begin** See the *BD FACSVerser System Reference* for the following procedures.

- Run performance QC.
- Run Assay & Tube Settings setup for the BD CBA kit tube settings.

### BD CBA kit workflow

The following table shows the typical stages of the BD CBA kit assay setup workflow.

Stage	Description
1	Running performance QC
2	Running Assay & Tube Settings setup
3	<a href="#">Preparing reagents (page 16)</a>
4	<a href="#">Confirming tube settings (page 17)</a>
5	<a href="#">Creating an assay from the standards experiment (page 20)</a>
6	<a href="#">Creating an assay from the samples experiment (page 21)</a>

### Preparing reagents

To prepare reagents:

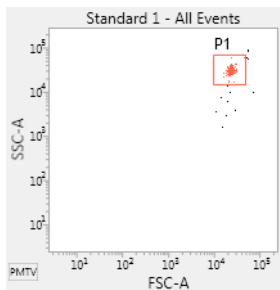
1. Add 100  $\mu$ L of cytometer setup beads, from your BD CBA kit, to a 12 x 75-mm tube.
2. Add 400  $\mu$ L of Wash Buffer from your BD CBA kit and vortex the tube.



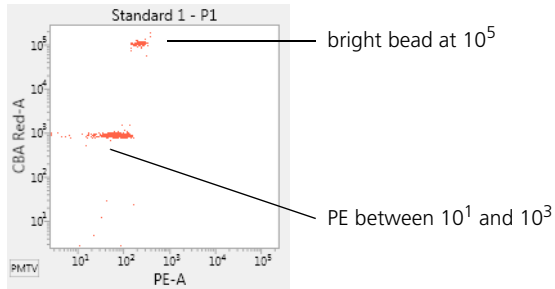
## Confirming tube settings

To confirm tube settings:

1. Open the BD CBA Kit Standards experiment.
2. Set the tube pointer to one of the tubes in the **Data Sources** panel.
3. Load the tube of BD CBA cytometer setup beads onto the cytometer and click **Preview**.
4. Confirm that the singlet bead population falls within the P1 gate.
  - If necessary, adjust the FSC and SSC voltages until the singlet bead population fits within P1.
  - You can use either the **PMT Voltages** panel or the data sliders on the dot plot to adjust the gain. Click **PMTV** to enable the sliders.



5. Confirm that the bright bead in the CBA Red channel falls at about  $10^5$ . If necessary, adjust the CBA Red voltage.



6. Confirm that the beads are falling between  $10^1$  and  $10^3$  in the PE channel. If necessary, adjust the PE voltage.
7. Click **Stop** and remove the tube of BD CBA cytometer setup beads.
8. Do one of the following:
  - If any changes were made to the tube settings, you will need to create new tube settings with a new name. Changes include the following: voltages, thresholds, window extensions, area scaling factors, and flow rate.
  - If adjustments were not made, you can use the tube settings that were imported with the setup experiments for your assay. Skip to [Creating an assay from the standards experiment \(page 20\)](#).

## Creating new tube settings

If any adjustments were made to the tube settings, then create new tube settings.

### To create new tube settings:

1. Right-click the tube that was previewed and select **Create Tube Settings**.

The **Create Tube Settings** wizard opens.

2. In the **CS&T lot ID** field, select the bead lot for your beads. This is the same bead lot as the one that you ran for the performance QC.
3. Place a tube of BD FACSuite CS&T beads on the cytometer.
4. Click **Acquire**.

When acquisition completes, the **Name and Description** dialog opens.

5. In the **Tube Settings name** field, type a name.

You can use a descriptive name or add a short description in the **Description** field to make your tube settings easier to identify.

6. Click **Finish**.

Your CBA kit user-defined tube settings are saved to the library.

7. Remove the tube of BD FACSuite CS&T beads from the cytometer.

## Applying new tube settings to the standard tubes

### To apply new tube settings to the standard tubes:

1. Shift+click the first and last standard tubes.

The imported setup experiment has 10 standard tubes. This selects and highlights all your tubes.

2. Right-click a tube and select **Properties** from the menu.

The **Tube Properties** dialog opens.

3. In the **General** tab, click **Select** in the **Tube Settings** field.

The **Select Tube Settings** dialog opens.

4. Select your CBA kit user-defined tube settings.
5. Click **Close**.

### Creating an assay from the standards experiment

Create an assay for the 10 standard tubes.

If you want to create replicates for the standards, then add the replicates as a worklist entry later in the setup. This will allow FCAP Array software to recognize them as replicates in the BD FACSuite workflow. See [Adding standards, samples, and controls to a worklist \(page 35\)](#).

**To create an assay from the standards experiment:**

1. Select **File > Create Assay**.

The **Create Assay** dialog opens.

2. In the **Name** field, type *BD CBA kit standards*.
3. (Optional) Select the **Share Assay** checkbox if you want this assay to be available to all users.
4. Click **OK**.

You can re-use the assay that you created if you run the same BD CBA kit or another single color BD CBA kit.

## Creating an assay from the samples experiment

Create an assay for the one sample tube.

There is no need to add tubes here for all your samples. If you add tubes to this assay, they will be identified by FCAP Array software as replicates in the BD FACSuite workflow. You will create worklist entries later in the setup for all your samples. See [Adding standards, samples, and controls to a worklist \(page 35\)](#).

### To create an assay from the samples experiment:

1. Open the BD CBA Kit Samples experiment.
2. (Optional) If you are running the same number of replicates for all tubes, then right-click a tube and select **Duplicate Without Data** to add your replicates. This will add tubes with the correct settings and parameters.
3. Apply the correct tube settings to the sample tube(s).
  - a. If you are running replicates, Shift+click the first and last tubes.
  - b. Right-click a tube and select **Properties** from the menu.
  - c. In the **General** tab, click **Select** in the **Tube Settings** field.

The **Select Tube Settings** dialog opens.

- d. If you created new tube settings for the standards, select your CBA kit user-defined tube settings. See [step 5 of Creating new tube settings \(page 19\)](#).

If you did not make any changes to the tube settings in the standards experiment, select the tube settings that imported with the standards experiment.

- e. Click **Close**.
4. Select **File > Create Assay**.

The **Create Assay** dialog opens.

5. In the **Name** field, type *BD CBA kit samples*.
6. (Optional) Select the **Share Assay** checkbox if you want this assay to be available to all users.
7. Click **OK**.

You can re-use the assay that you created if you run the same BD CBA kit or another single color BD CBA kit.

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**Next step**

Proceed to [Adding standards, samples, and controls to a worklist \(page 35\)](#).

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## Running assay setup for BD CBA flex sets

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### Introduction

This topic describes how to confirm the BD CBA flex set tube settings that were imported as part of the setup experiments and how to create reference settings for your BD CBA flex set assay using BD FACSuite software. Also, you will create assays from the standards and samples setup experiments.

Reference settings must be updated every 30 days.

**Before you begin** See the *BD FACSVerser System Reference* for the following procedures:

- Run performance QC.
- Run Assay & Tube Settings setup for the BD CBA flex set tube settings.

**BD CBA flex set workflow** The following table shows the typical stages of the BD CBA flex set assay setup workflow.

Stage	Description
1	Running performance QC
2	Running Assay & Tube Settings setup
3	<a href="#">Preparing setup reagents (page 23)</a>
4	<a href="#">Confirming tube settings (page 24)</a>
5	<a href="#">Setting the stopping rule (page 26)</a>
6	<a href="#">Adjusting compensation (page 27)</a>
7	<a href="#">Applying new tube and reference settings to the standard tubes (page 29)</a>
8	<a href="#">Creating an assay from the standards experiment (page 30)</a>
9	<a href="#">Creating an assay from the samples experiment (page 31)</a>

**Preparing setup reagents** To prepare setup reagents:

1. Label four 12 x 75-mm tubes: A9, PE-F1, CBA Red, and CBA NIR.
2. Add 300  $\mu$ L of Wash Buffer from your Master Buffer Kit to each tube.
3. Vortex the stock vials of setup beads in your Master Buffer Kit.

4. Add setup beads to the tubes as follows.
    - A9: Add 50  $\mu$ L of Instrument Setup Bead A9.
    - PE-F1: Add 50  $\mu$ L of PE Instrument Setup Bead F1.
    - CBA Red: Add 50  $\mu$ L of Instrument Setup Bead F1 and 50  $\mu$ L of Instrument Setup Bead F9.
    - CBA NIR: Add 50  $\mu$ L of Instrument Setup Bead F1 and 50  $\mu$ L of Instrument Setup Bead A1.
- 

**Confirming tube settings**

You need to run tubes A9 and PE-F1 to confirm the tube settings.

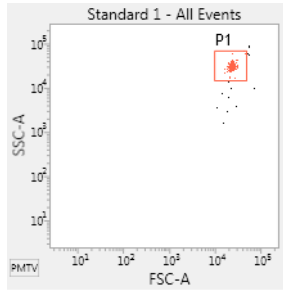
**To confirm tube settings:**

1. Open the BD CBA Flex Set Standards experiment.
2. Load the A9 tube onto the cytometer.
3. Set the current tube pointer on a tube and click **Preview**.
4. Confirm that the singlet bead population falls within the P1 gate.

If necessary, adjust the FSC and SSC voltages until the singlet bead population fits within P1.



You can use either the **PMT Voltages** panel or the data sliders on the dot plot to adjust the gain. Click **PMTV** to enable the sliders.

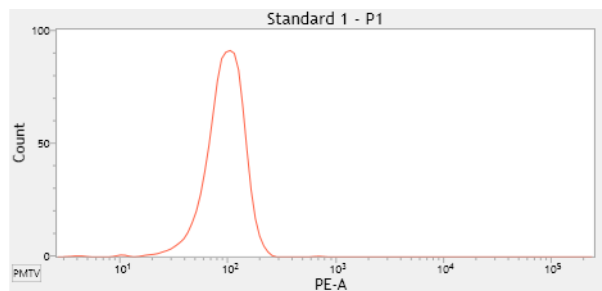


- Confirm that CBA Red and CBA NIR medians fall within 130,000 to 190,000.

Statistics

Name	Events	% Parent	% Grandparent	% Total	FSC-A Mean	SSC-A Mean	CBA Red-A Median	CBA NIR-A Median	PE-A Median
Standard 1:P1	344	98.29	##	98.29	31,604	30,903	160,587	161,250	724

- Click **Stop**, remove the A9 tube, and load the PE-F1 tube.
- Click **Preview**.
- Confirm that the beads are falling between  $10^1$  and  $10^3$  in the PE channel. If necessary, adjust the PE voltage.



9. Click **Stop** and remove the PE-F1 tube.
- 

### Setting the stopping rule

The stopping rule needs to be adjusted to accommodate the size of your plex.

#### To set the stopping rule:

1. Shift+click the first and last tubes.  
This selects and highlights all your tubes.
2. Right-click and select **Properties**.  
The **Tube Properties** dialog opens.
3. Click the **Acquisition** tab.
4. Set the singlet (P1) gate as the storage gate.
5. Under **Create Gate Criteria**, do the following:
  - a. Set the singlet (P1) gate as the stopping gate.
  - b. Set events to record to 300 events per analyte (eg,  $300 \times 6 = 1,800$  events for a 6 plex).
6. Click **Add Criteria**.
7. Under the **Combine Gate Criteria and Apply Rule** field, click the new event criteria you just defined.
8. Click **Apply Rule**.
9. Click **Close**.
10. In the **Acquisition Status** panel, set **Events to Display** to 5,000.

## Adjusting compensation

You need to run the BD FACSuite CS&T research beads, and CBA Red and CBA NIR tubes to adjust compensation.

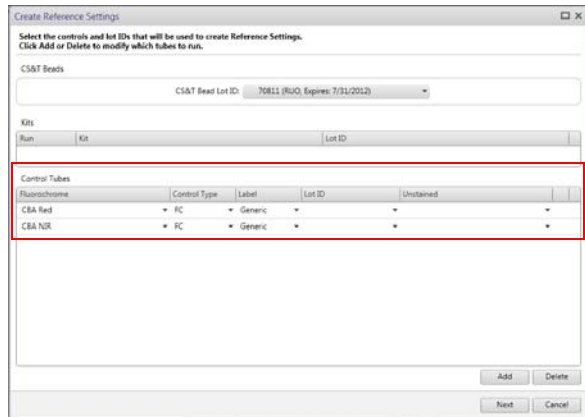
### To perform compensation adjustments:

1. Right-click the tube that was previewed and select **Create Reference Settings**.

The **Create Reference Settings** wizard opens.

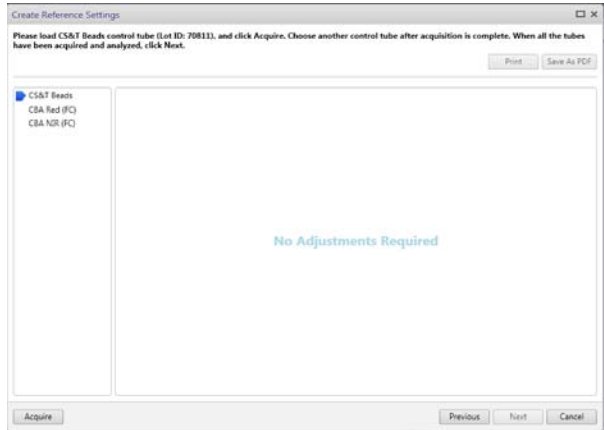
**Note:** If the tubes are not displayed in the list of Control Tubes when the wizard opens, click **Add** to add tubes for the CBA Red and CBA NIR fluorochromes.

2. Set up both CBA Red and CBA NIR tubes in the following way:
  - CBA Red / NIR (Control Type: FC; Label: Generic; Lot ID: blank; Unstained: blank)



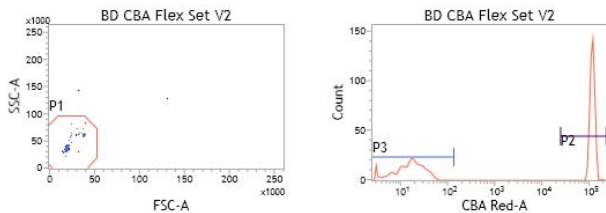
3. In the **CS&T Bead Lot ID** field, select the bead lot for your beads. This is the same bead lot as the one that you ran for the performance QC.

4. Click Next.

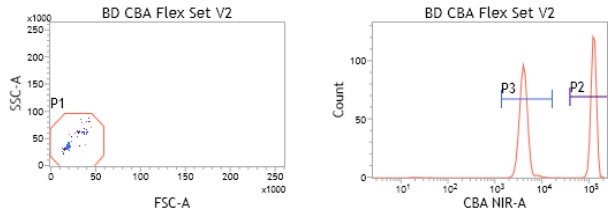


5. Acquire the CS&T Beads tube. No plots will be shown while this tube is running. Once the CS&T tube completes, a green check mark will be displayed by that tube.
6. Acquire the CBA Red tube. Move the gates as shown in the figure to capture each of the populations.

**Note:** The first time a compensation tube (CBA Red or CBA NIR) is run, the populations will shift while the instrument confirms the voltage settings. Wait until the populations stabilize before setting the gates.



7. Acquire the CBA NIR tube. Move the gates as shown in the figure to capture each of the populations.



8. Once all three tubes (CS&T Beads, CBA Red, and CBA NIR) have completed successfully, click **Next**.

**Note:** If an error message is displayed stating that there are not enough events in the gate, you may go back and widen that gate to try to capture enough events.

9. In the **Reference Settings Name** field, type a name for your CBA flex set user-defined tube settings and reference settings.

You can use a descriptive name or add a short description in the **Description** field to make your tube and reference settings easier to identify.

10. Click **Finish**.

### Applying new tube and reference settings to the standard tubes

#### To apply new tube and reference settings:

1. Shift-click the first and last standard tubes.  
The imported setup experiment has 10 standard tubes. This selects and highlights all your tubes.
2. Right-click a tube and select **Properties** from the menu.

The **Tube Properties** dialog opens.

3. In the **General** tab, click **Select** in the **Tube Settings** field.

The **Select Tube Settings** dialog opens.

4. Select your CBA flex set user-defined tube settings and reference settings. See [step 9 of Adjusting compensation \(page 27\)](#).
5. Click **Close**.
6. (If applicable) For the BD CBA Enhanced Sensitivity assays, delete Standard 9 and Standard 10 from the experiment before creating an assay in BD FACSuite software.

### Creating an assay from the standards experiment

Create an assay for the 10 standard tubes.

If you want to create replicates for the standards, then add the replicates as a worklist entry later in the setup. This allows FCAP Array software to recognize them as replicates in the BD FACSuite workflow. See [Adding standards, samples, and controls to a worklist \(page 35\)](#).

**To create an assay from the standards experiment:**

1. Select **File > Create Assay**.

The **Create Assay** dialog opens.

2. In the **Name** field, type *BD CBA flex set standards*.

You can append to the assay name above or create your own names if you are using several different sized multiplexes or BD CBA flex sets.

3. (Optional) Select the **Share Assay** checkbox if you want this assay to be available to all users.
4. Click **OK**.

You can re-use the assay that you created if the reference settings are still valid and you run the same BD CBA flex set or a BD CBA flex set multiplex of the same size.

## Creating an assay from the samples experiment

Create an assay for the one sample tube.

There is no need to add tubes here for all your samples. If you add tubes to this assay, they will be identified by FCAP Array software as replicates in the BD FACSuite workflow. You will create worklist entries later in the setup for all your samples. See [Adding standards, samples, and controls to a worklist \(page 35\)](#).

**To create an assay from the samples experiment:**

1. Open the BD CBA Flex Set Samples experiment.
2. Right-click the tube and select **Properties** from the menu.

The **Tube Properties** dialog opens.

3. In the **General** tab, click **Select** in the **Tube Settings** field.

The **Select Tube Settings** dialog opens.

4. Select your CBA flex set user-defined tube and reference settings. See [step 9 of Adjusting compensation \(page 27\)](#).
5. Set the stopping rule. See [step 3 through step 8 of Setting the stopping rule \(page 26\)](#).
6. Click **Close**.
7. (Optional) If you are running the same number of replicates for all tubes, then right-click a tube and select **Duplicate Without Data** to add your replicates. This will add tubes with the correct settings and parameters.

8. Select **File > Create Assay**.

The **Create Assay** dialog opens.

9. In the **Name** field, type *BD CBA flex set samples*.

You can append to the assay name above or create your own names if you are using several different sized multiplexes or BD CBA flex sets.

10. (Optional) Select the **Share Assay** checkbox if you want this assay to be available to all users.

11. Click **OK**.

You can re-use the assay that you created if the reference settings are still valid and you run the same BD CBA flex set or a BD CBA flex set multiplex of the same size.

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**Next step**

Proceed to [Setting FCS export options \(page 34\)](#).

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## Preferences and keywords

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The chapter covers the following topics:

- [Setting FCS export options \(page 34\)](#)
- [Adding standards, samples, and controls to a worklist \(page 35\)](#)
- [BD CBA keywords overview \(page 38\)](#)
- [Assigning values to BD CBA keywords \(page 40\)](#)

## Setting FCS export options

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**Introduction** This topic explains how to set your FCS export options in the **Preferences** dialog of BD FACSuite software. FCS file export is required for analysis in FCAP Array software.

Set your export options now so they will be active for the worklist you will create in the next section.

- 
- Procedure** To set FCS export options:
1. From the menu bar, select **Tools > Preferences**.  
The **Preferences** dialog opens.
  2. Click the **Worklists** tab.
  3. Select **Export > FCS**.
  4. Select the **Export FCS after acquisition** checkbox.
  5. (Optional) Select an export folder location. The default location is C:\BD Export\FCS\Worklists.
  6. Click **OK**.

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**Next step** Proceed to [Adding standards, samples, and controls to a worklist \(page 35\)](#).

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## Adding standards, samples, and controls to a worklist

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**Introduction** This topic describes how to create a worklist, including adding entries and selecting loading options.

We recommend that you acquire standards, samples, and controls in a worklist.

### Adding standards to the worklist

Standards should be acquired in order of increasing concentration, starting with the 0-pg/mL tube.

#### To add standards to the worklist:

1. In the **Worklists** workspace, select **File > New Worklist**.
2. Click in the **Task** column and select *BD CBA kit standards UD* or *BD CBA flex set standards UD* (depending on the reagents you are using).
3. In the first blank row in the worklist, click the **Sample ID** column, then type a sample ID for your BD CBA standards.

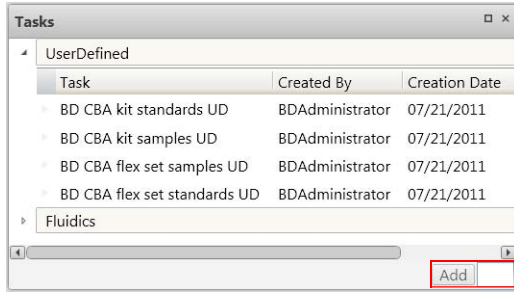
If you are running replicate standards, repeat steps 2 and 3. You must type the exact same sample ID for each replicate entry. FCAP Array software interprets standards with the same sample ID and CBA Standard ID as replicates.

### Adding samples to the worklist

#### To add samples to the worklist:

1. In the **Tasks** panel, expand the **UserDefined** list.
2. Select *BD CBA kit samples UD* or *BD CBA flex set samples UD* (depending on the reagents you are using) from the **Task** column.

- In the field next to the **Add** button, type the number of samples you want to add to the worklist.



- Click **Add**.  
A worklist entry is created for each of your samples.
- Type a sample ID for each entry.

### Adding controls to the worklist

#### To add controls to the worklist:

- In the **Tasks** panel, expand the **UserDefined** list.
- Select *BD CBA kit samples UD* or *BD CBA flex set samples UD* (depending on the reagents you are using) from the **Task** column.
- In the field next to the **Add** button, type the number of controls you want to add to the worklist.
- Click **Add**.  
A worklist entry is created for each of your controls.
- Type a sample ID for each entry.

---

**Selecting a loading option**

As you add entries in a worklist, each tube in the entry is added to the layout view based on the loading options and run pattern you selected when you defined the layout. Settings can be changed in Loader preferences. See the *BD FACSVerse System Reference*.

The default run pattern for the Loader is linear, horizontal, use edge positions, and no empty positions between tubes or entries.

**To select a loading option:**

1. In the **Loading Options** panel, select **Manual** or **UAL**.

**Note:** If the BD FACS Universal Loader is being used with filter plates, you must use Millipore Catalog No. MABVN12. This plate is different than the one recommended in the BD CBA reagent manuals.

2. In the **Loading Options** panel, select your **Carrier Type**.

---

**Next step**

Proceed to [Assigning values to BD CBA keywords \(page 40\)](#).

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## BD CBA keywords overview

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### Introduction

This topic describes keywords used when you run BD CBA assays with BD FACSuite software. These keywords are written to the FCS file and are used by FCAP Array software to optimize your workflow and minimize repetitive data entry.

See the FCAP Array software documentation for more information.

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### Keywords

Keywords provide information used in plex creation in FCAP Array software. This in turn optimizes the workflow.

Some keywords are used to generate file names. Use characters that are valid for Windows file names.

A file name cannot contain any of the following characters:  
\\ : \* ? " < > |

FCAP Array software identifies your files using the worklist or experiment name from BD FACSuite software. Assign unique names for each run (ie, Human\_TH1-TH2\_Kit\_1-1-2011).

FCAP Array software identifies samples as replicates when they have the same sample ID in BD FACSuite software. Replicate standards have both the same sample ID and CBA Standard ID keyword. Replicate controls have the same CBA Control ID keyword only. See

[Adding standards to the worklist \(page 35\)](#) for more information.

Keyword	Description
CBA Plex Name	<ul style="list-style-type: none"> <li>● Identifies the specific plex from the FCAP Array software plex library. If a match is found in the plex library, the FCS files are automatically associated with that plex. The first time a plex is used it will have to be created in FCAP Array software.</li> <li>● Use characters that are valid in a Windows file name to ensure the plex will be exportable in FCAP Array software.</li> </ul>
CBA Type	<ul style="list-style-type: none"> <li>● Identifies whether the tube or well contains a standard, sample, or control.</li> <li>● FCAP Array software lists analysis results in the following order: standards, samples, controls.</li> </ul>
CBA Standard ID	<ul style="list-style-type: none"> <li>● Identifies the number of the standard contained in the tube or well. The value can also be Pos or Neg to identify positive and negative for qualitative BD CBA assays.</li> <li>● Standards will be arranged for the standard curve in alphabetical order by the value of the CBA Standard ID keyword. Assign this keyword appropriately to ensure that standards are plotted in the correct order on the standard curve.</li> <li>● Use leading zeros for the CBA Standard ID keyword. This will ensure that your files can always be sorted in order (01, 02, 03 as opposed to 1, 2, 3).</li> </ul>
CBA Control ID	<ul style="list-style-type: none"> <li>● Identifies the control contained in the tube or well.</li> <li>● Controls will be arranged in alphabetical order by the value of the CBA Control ID keyword.</li> </ul>
CBA Dilution	<ul style="list-style-type: none"> <li>● Used to specify the dilution of the sample.</li> <li>● The default dilution value is 1.00.</li> <li>● Note that the dilution value can be entered in BD FACSuite software or FCAP Array software.</li> </ul>

## Assigning values to BD CBA keywords

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**Introduction** This topic describes how to assign values to the BD CBA keywords.

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**Assigning values to keywords** CBA Type and CBA Standard ID have values assigned to them in the imported setup experiments. You might need to assign values to CBA Plex Name, CBA dilution, and CBA Control ID.

### To assign values to keywords:

1. Expand the entries to show all the tubes in the worklist.
2. Click in a field under a keyword column and type a keyword value.

See [Keywords \(page 38\)](#) for more information.

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**Next step** Acquire the worklist entries according to the instructions in the *BD FACSVersé System Reference* and then analyze your data according to the instructions in the *FCAP Array Software Version 3.0 User's Guide*.

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