BD FACSDiva Software Quick Reference Guide for BD FACSCanto Systems with Loader Option

This guide contains instructions for using BD FACSDiva™ software version 6.0 and later with BD FACSCanto and BD FACSCanto II systems equipped with the BD FACS™ Loader option.

Workflow Overview

The following figure shows the steps for daily workflow using BD FACSDiva software.

Start Up System ▶ Check Performance ▶ Set Up Experiment ▶ Record Data ▶ Analyze Data ▶ Shut Down System

Before starting your daily workflow, ensure that your lab's software administrator has performed all the necessary tasks to set up the software for your use. This guide shows a workflow that uses application settings.

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Starting Up the System

1. Turn on the cytometer main power.

2. Start up the computer, start BD FACSDiva software, and log in.

3. Check fluid levels in the Cytometer window.

4. Select Cytometer > Fluidics Startup if automatic cleaning is disabled.

5. Check the flow cell for air bubbles.

6. Check that laser warmup has finished, indicated by a ready status.

The system is ready

Checking Cytometer Performance

1. Select Cytometer > CST.

Verify the Cytometer Configuration and bead Lot ID.

Clear the checkbox.

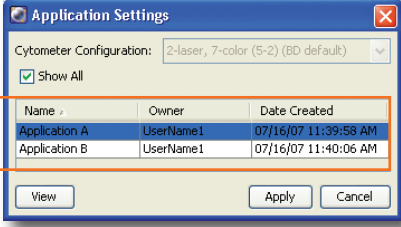If needed, select a different configuration or bead lot ID.

2. Place a tube of the BD™ Cytometer Setup and Tracking beads in position 1 on a carousel and run the beads.

3. View the Cytometer Performance Report.

4. Close the Cytometer Setup and Tracking window.
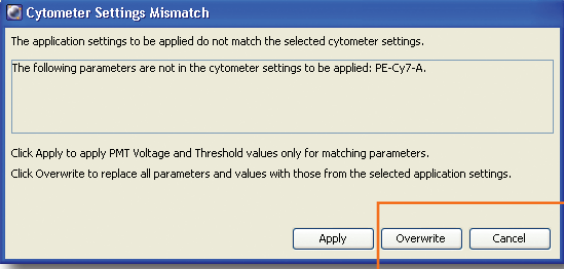
23-9548-00

Setting Up the Experiment

1 Select Edit > User Preferences and verify that selected preferences are appropriate.

2 Create an experiment in the Browser.

3 Right-click [Cytometer Settings] in the Browser. Select Application Settings > Apply.
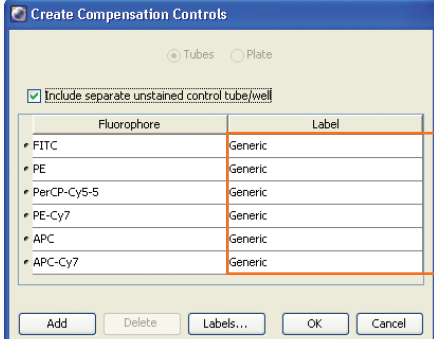
Application Settings

Cytometer Configuration: 2-laser, 7-color (5-2) (BD default)

☑ Show All

Name	Owner	Date Created
Application A	UserName1	07/16/07 11:39:58 AM
Application B	UserName1	07/16/07 11:40:06 AM

Select an application setting.

View   Apply   Cancel

Cytometer Settings Mismatch

The application settings to be applied do not match the selected cytometer settings.

The following parameters are not in the cytometer settings to be applied: PE-Cy7-A.

Click Apply to apply PMT Voltage and Threshold values only for matching parameters.
Click Overwrite to replace all parameters and values with those from the selected application settings.

Apply   Overwrite   Cancel

Click Overwrite if necessary.

4 Select Experiment > Compensation Setup > Create Compensation Controls.

Create Compensation Controls

◉ Tubes   ○ Plate

☑ Include separate unstained control tube/well
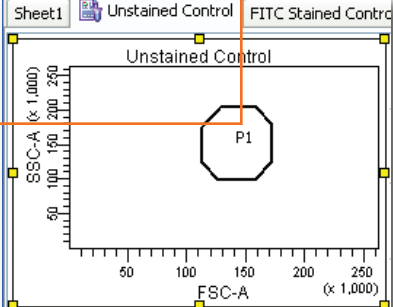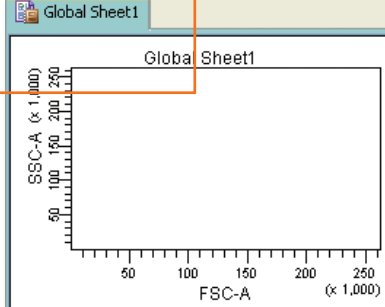
Fluorophore	Label
FITC	Generic
PE	Generic
PerCP-Cy5-5	Generic
PE-Cy7	Generic
APC	Generic
APC-Cy7	Generic

Create label-specific controls as needed.
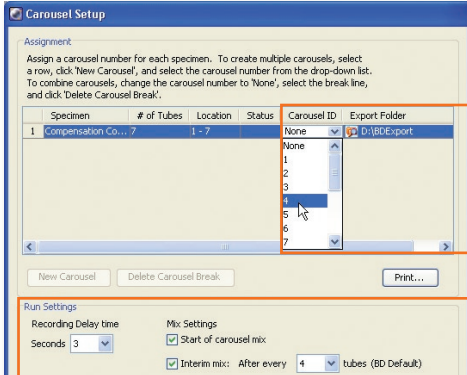
Add   Delete   Labels...   OK   Cancel

5 Copy and paste plots from the Unstained Control normal worksheet to a Global Worksheet.

Select Edit > Select All and then Edit > Copy to copy plots from the normal workseet.

Sheet1   Unstained Control   FITC Stained Contro

Unstained Control
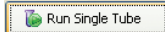
P1

Select Edit > Paste to paste the plots to a global worksheet.

Global Sheet1

Global Sheet1

6 Specify Carousel Setup settings.

Carousel Setup

Assignment

Assign a carousel number for each specimen. To create multiple carousels, select a row, click 'New Carousel', and select the carousel number from the drop-down list. To combine carousels, change the carousel number to 'None', select the break line, and click 'Delete Carousel Break'.
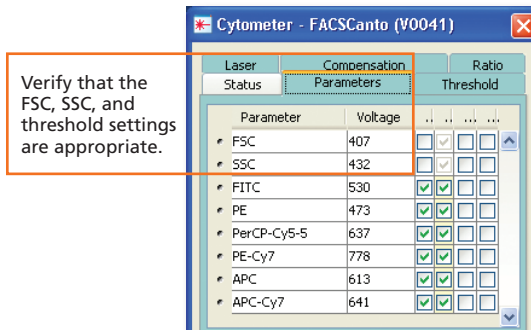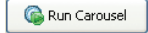
Specimen	# of Tubes	Location	Status	Carousel ID	Export Folder
1 Compensation Co...	7	1 - 7		None	D:\BDExport

None
1
2
3
4
5
6
7

Specify a carousel ID.

New Carousel   Delete Carousel Break   Print....

Run Settings

Recording Delay time   Mix Settings
Seconds 3   ☑ Start of carousel mix
☑ Interim mix: After every 4 tubes (BD Default)
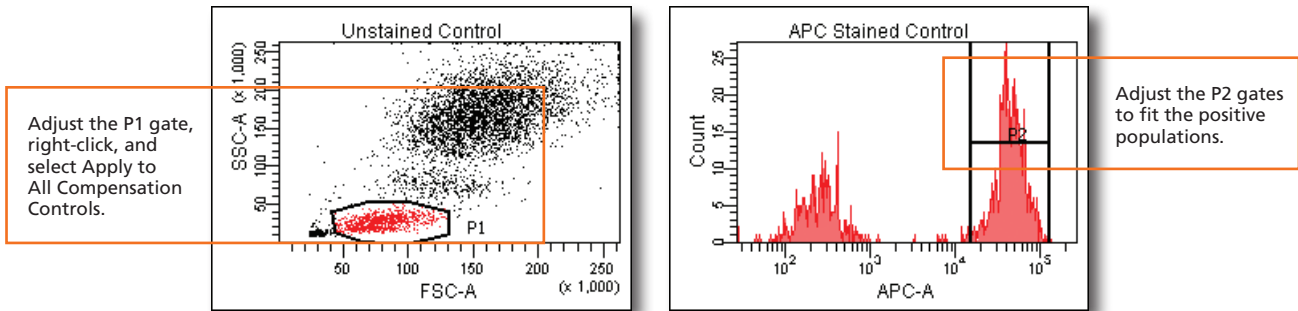
Specify run settings.

7 Verify that the current tube pointer is set to the Unstained Control tube and that a global worksheet is displayed.

8 Install compensation control tubes in the carousel in the same order as listed in the Browser and install the carousel on the Loader.

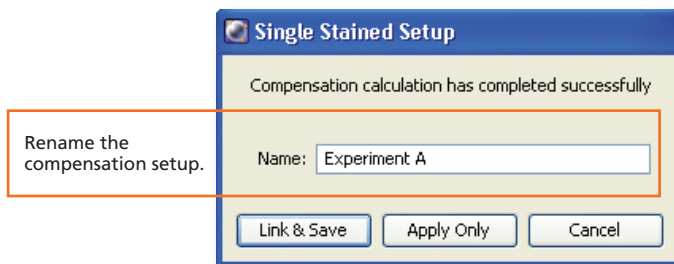**9** Verify that the cytometer is configured for automatic loading and Click [Run Single Tube] .

Select a tube to load.

4
Carousel

Verify that the FSC, SSC, and threshold settings are appropriate.

10 Click [Unload] and then click [Run Carousel] .
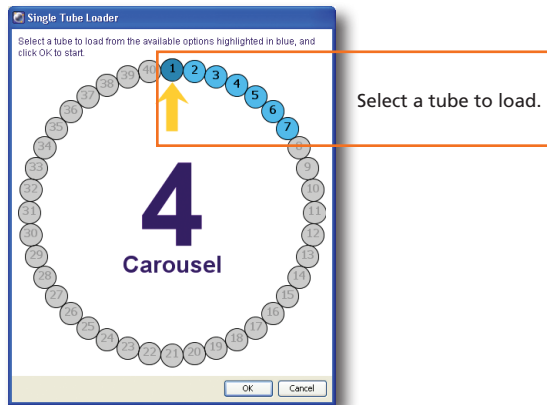
11 View recorded data in the normal worksheets and gate the positive populations.

Adjust the P1 gate, right-click, and select Apply to All Compensation Controls.

Adjust the P2 gates to fit the positive populations.

12 Select Experiment > Compensation Setup > Calculate Compensation.

Rename the compensation setup.

Recording Specimen Data

1 Create Browser elements.

Use the Browser toolbar to add elements.

2 Specify Carousel Setup settings.

Specify a carousel ID.
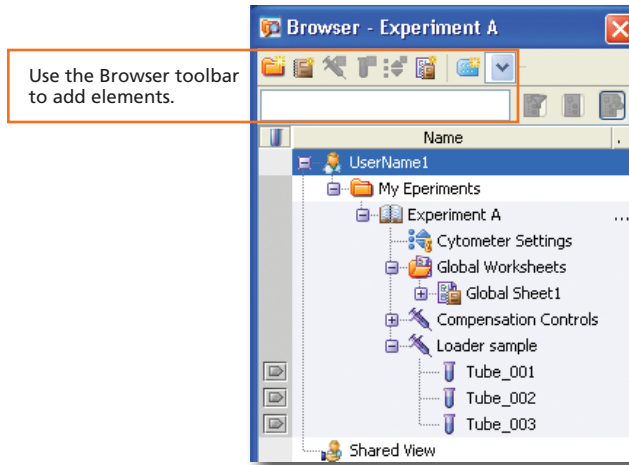
Specify run settings and tube pressurization error handling settings.
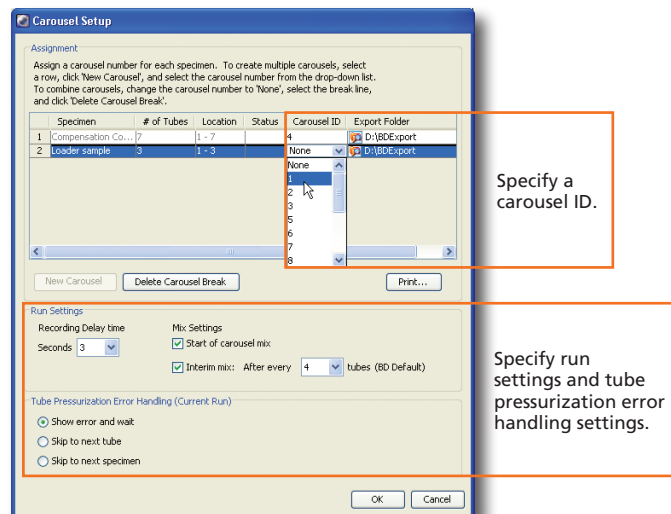
3 Make entries in the Experiment Layout.

Specify reagents labels, keywords, and acquisition criteria as needed.
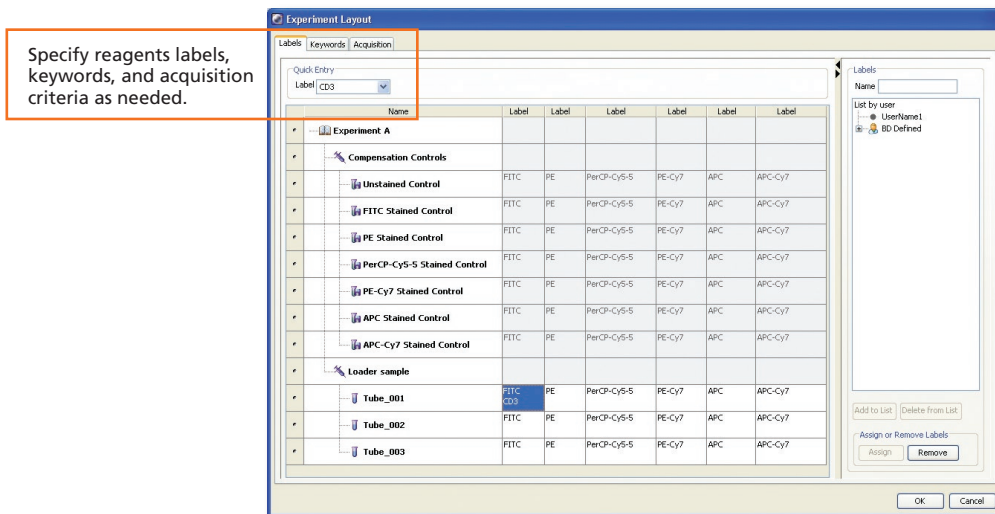
4 Install the carousel on the Loader and click **Run Carousel** .
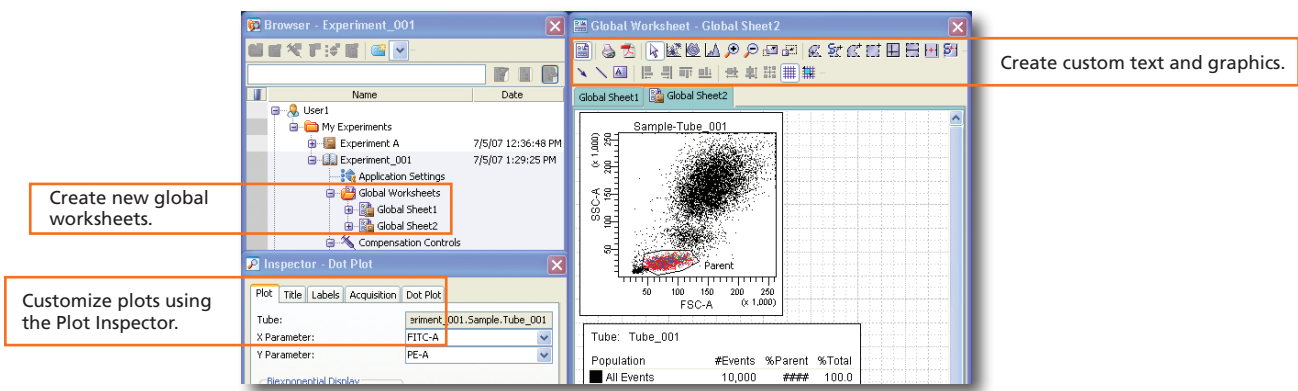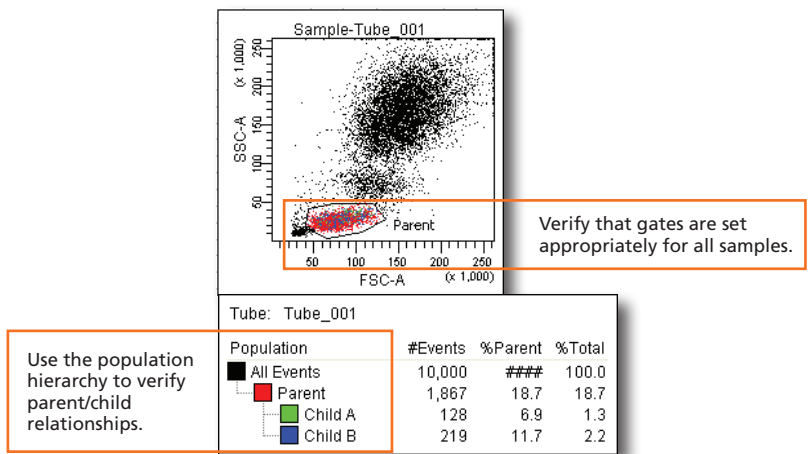
5 Specify tubes to run.
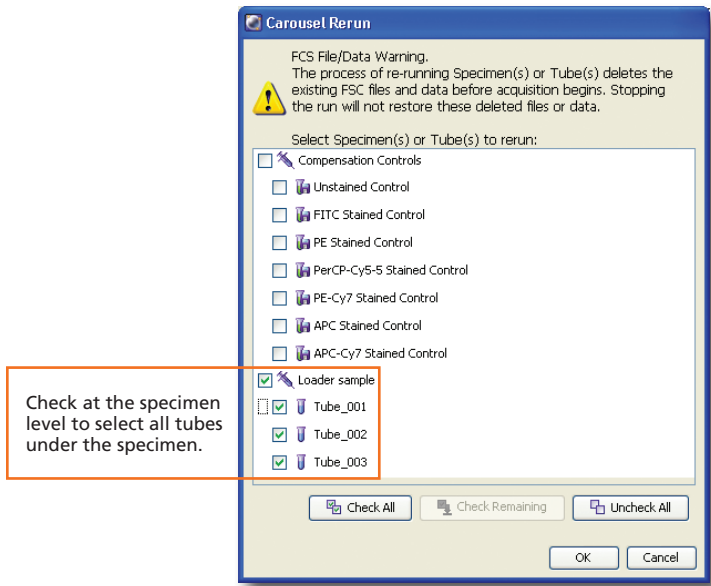
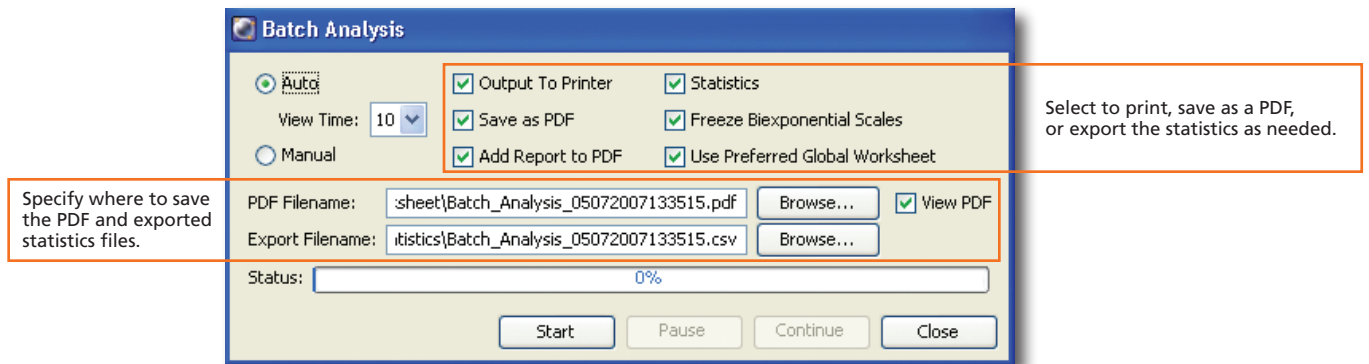Check at the specimen level to select all tubes under the specimen.

Analyzing Data

1 Create plots, gates, and statistics needed for analysis.

Create new global worksheets.

Customize plots using the Plot Inspector.

Create custom text and graphics.

2 Perform quality control of the analysis.

Verify that gates are set appropriately for all samples.

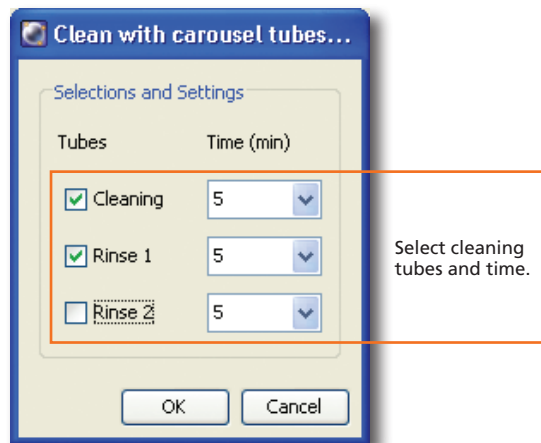Use the population hierarchy to verify parent/child relationships.

3 Do one of the following to print or export the results.

- Select File > Print to print the active worksheet.
- Select File > Export to export selected elements.
- Right-click a specimen or experiment and select Batch Analysis (using a global worksheet).

Select to print, save as a PDF, or export the statistics as needed.

Specify where to save the PDF and exported statistics files.

Shutting Down the System

1 Select Carousel > Clean.

Select cleaning tubes and time.

2 Install the carousel with the appropriate cleaning tubes and perform the cleaning cycle.

3 Perform a fluidics shutdown.

4 Empty waste and refill fluids if prompted to do so.

5 Turn off the cytometer main power and shut down the computer.