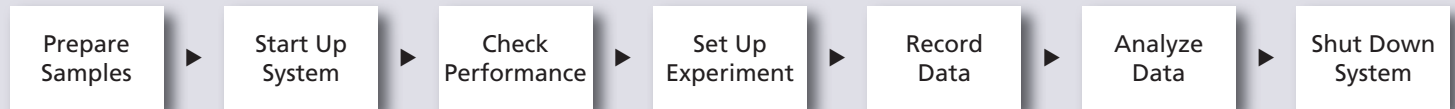


BD FACSDiva Software Quick Reference Guide for BD FACSCanto Systems with Loader Option

This guide contains instructions for using BD FACSDiva™ software version 6 with BD FACSCanto™ and BD FACSCanto II systems equipped with the BD FACSTM Loader option. The workflow shown uses the BD FACSTM Sample Prep Assistant III (SPA III) and BD FACSTM Lyse Wash Assistant (LWA) to prepare lyse/wash samples. The workflow shown also uses application settings in BD FACSDiva software. Before starting your daily workflow, ensure that your lab's software administrator has performed all the necessary tasks to set up the SPA III, LWA, and BD FACSDiva software for your use.

Workflow Overview

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

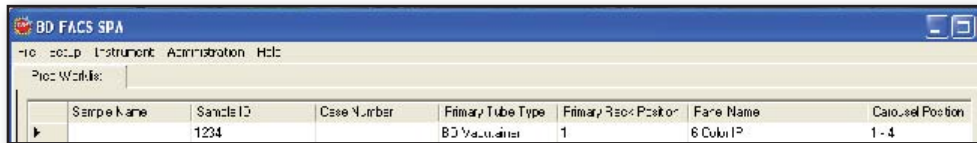


BD

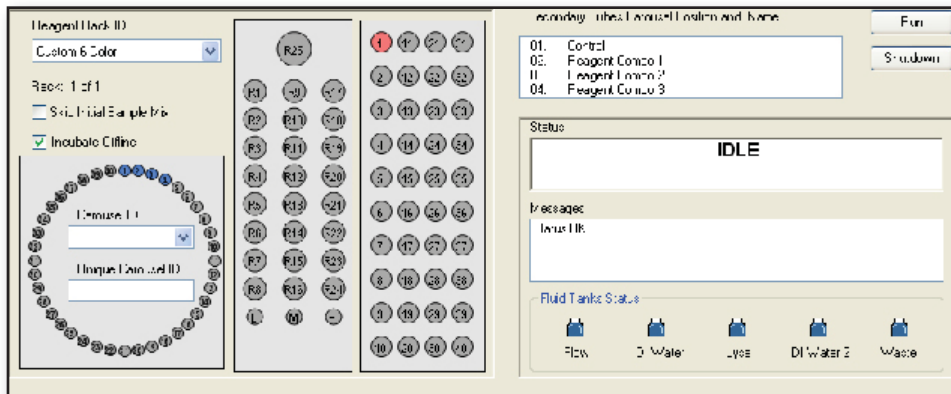
Helping all people
live healthy lives

Preparing Samples

- 1 Perform daily inspection and startup for the SPA III and startup for the LWA.
- 2 Set up the worklist in the BD FACSTM SPA software.



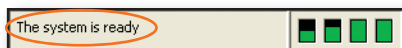
- 3 Load the primary tube rack, secondary tubes into the carousel, and reagents into the reagent rack as specified in the software.



- 4 Close the safety cover and click **Run** to process samples on the SPA III.
- 5 Save and print the SPA worklist.
- 6 Transfer the SPA worklist to a location where it can later be imported into BD FACSDiva software.
- 7 Transfer the carousel from the SPA III to the LWA.
- 8 Run the appropriate LWA protocol.
- 9 Perform daily cleaning for the SPA III and LWA.
- 10 Shut down the SPA III and LWA.

Starting Up the System

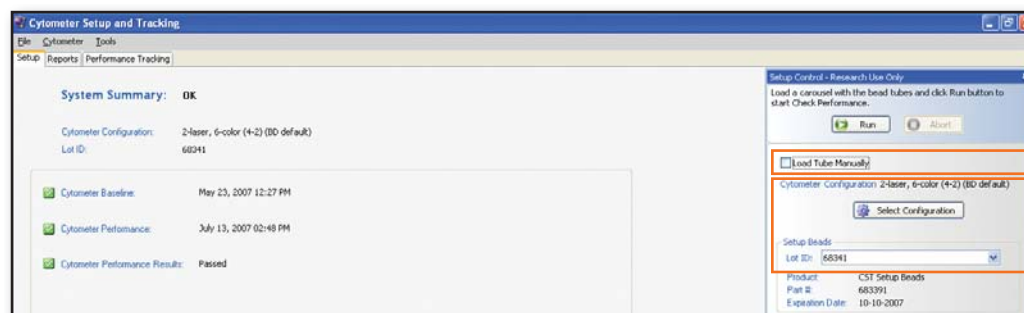
- 1 Turn on the cytometer main power.
- 2 Start up the computer, start BD FACSDiva software, and log in.
- 3 Check fluid levels in the **Cytometer** window.
- 4 Select **Cytometer > Fluidics Startup** if automatic cleaning is disabled.
- 5 Check the flow cell for air bubbles.
- 6 Check that laser warmup has finished, indicated by a ready status.



TIP Allow the lasers to warm up for 15 to 30 minutes before running samples on the cytometer to ensure laser stability and optimal power.

Checking Cytometer Performance

- 1 Select **Cytometer > CST**.



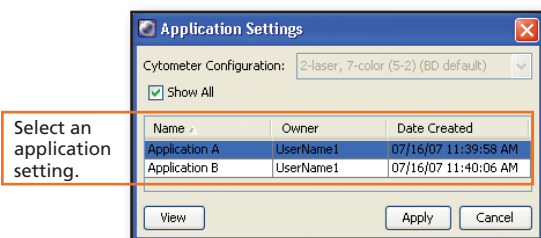
Clear this checkbox.

Verify the Current Configuration and bead Lot ID.

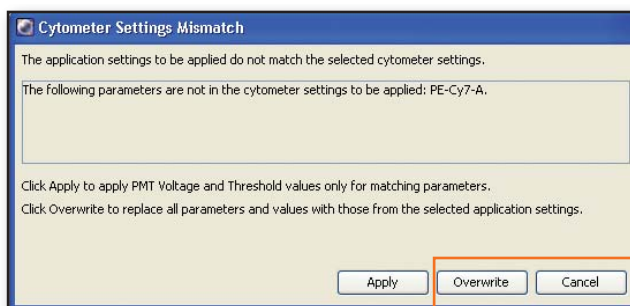
- 2 Place a tube of the BD™ Cytometer Setup and Tracking beads* in position 1 on a carousel and run the beads.
- 3 View the Cytometer Performance Report.
- 4 Close the **Cytometer Setup and Tracking** window.

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 in the Browser. Select **Application Settings > Apply**.

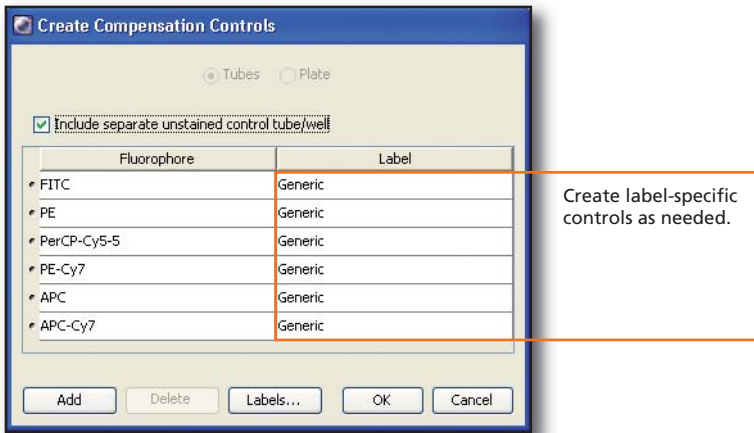


Select an application setting.

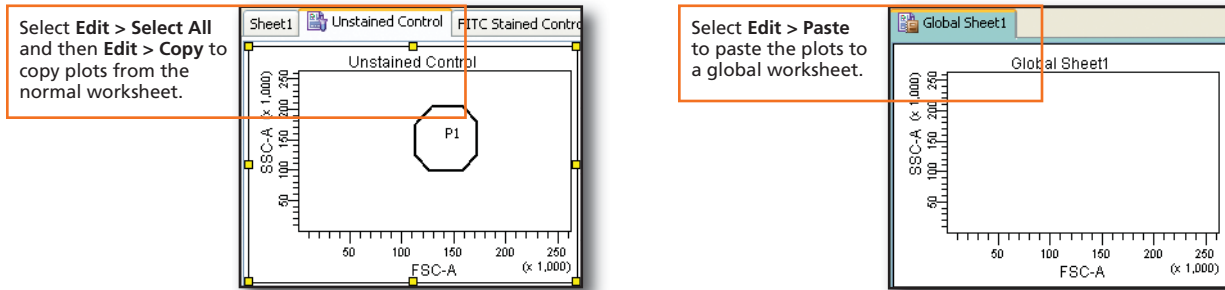


Click **Overwrite** if necessary.

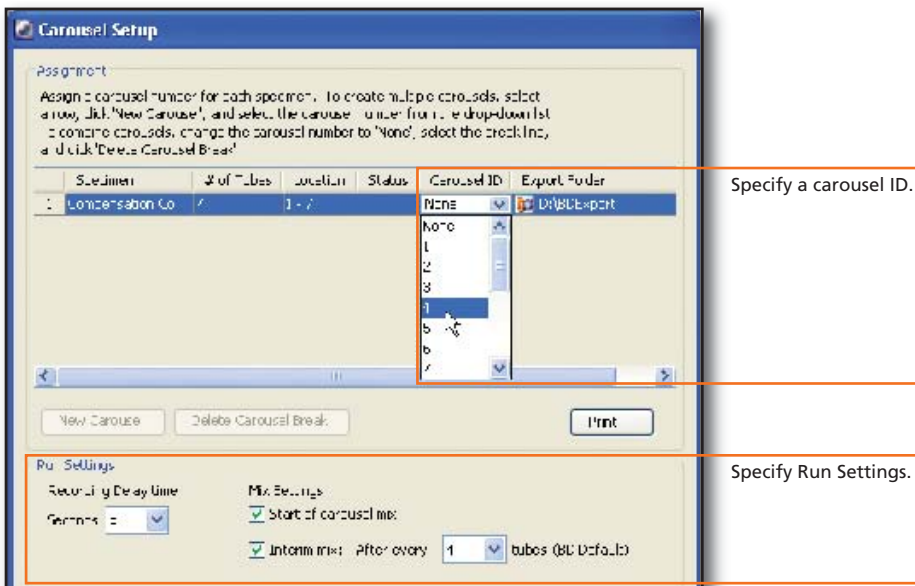
- Select **Experiment > Compensation Setup > Create Compensation Controls**.



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



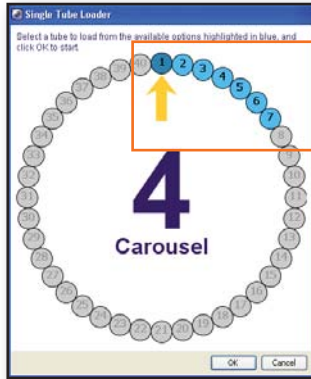
- Specify **Carousel Setup** settings.



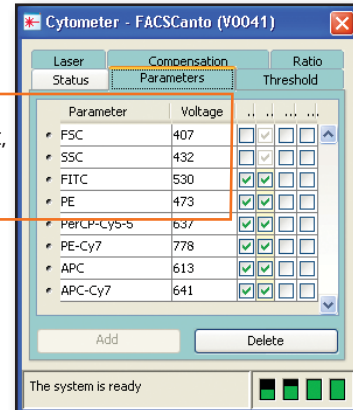
- Verify that the current tube pointer is set to the Unstained Control tube and a global worksheet is displayed.
- If using compensation beads, write down the FSC, SSC, and threshold values displayed in the **Cytometer** window.
- Place compensation control tubes in the carousel in the same order listed in the Browser, and install the carousel in the Loader.

- 10 Verify that the cytometer is configured for automatic loading and that settings are appropriate for the compensation controls.

Application settings are optimized for cellular samples. You might need to adjust settings for compensation controls prior to recording data for controls.



Select a tube to load and click **Run Single Tube**.

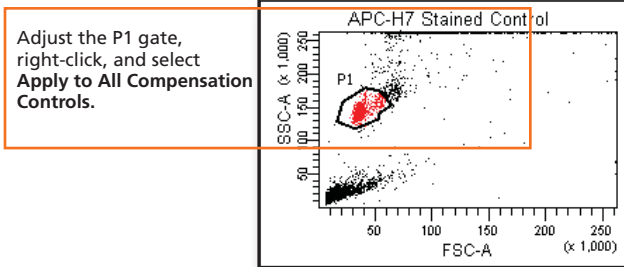


Verify that the FSC, SSC, and threshold settings are appropriate.

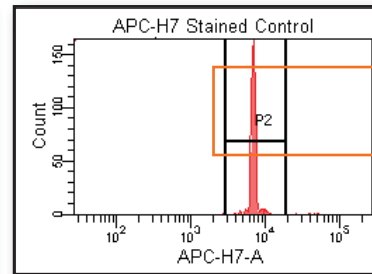
- 11 Click Unload and then click Run Carousel.

- 12 View the Carousel Report and check for any error messages.

- 13 View recorded data in the normal worksheets and gate the positive populations.

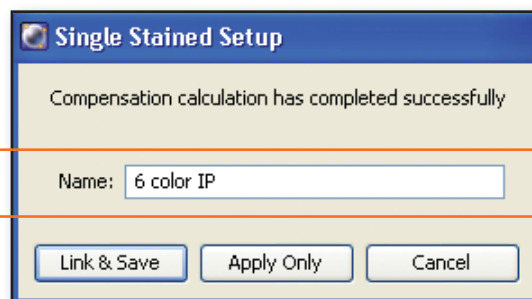


Adjust the P1 gate, right-click, and select **Apply to All Compensation Controls**.



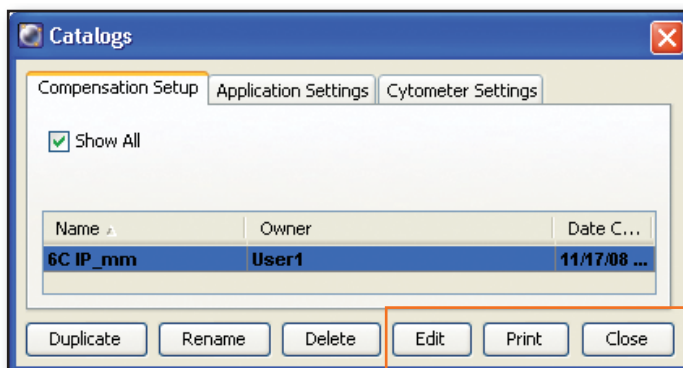
Adjust the P2 gates to fit the positive populations.

- 14 Select **Experiment > Compensation Setup > Calculate Compensation**.



Rename the compensation setup.

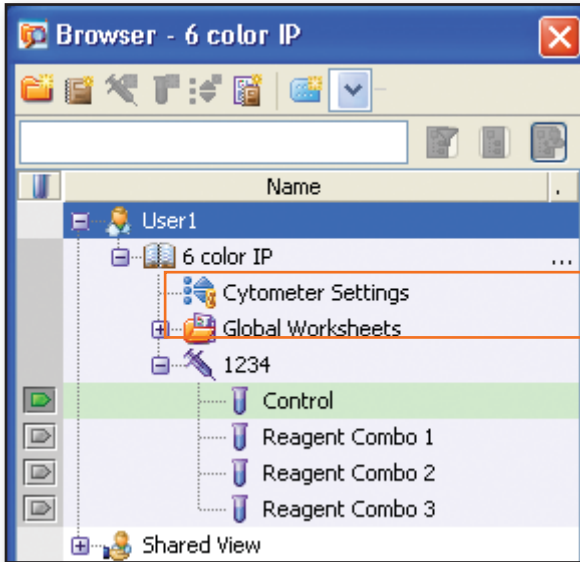
- 15 If needed, select **Cytometer > Catalogs** and return the FSC, SSC, and threshold settings to values appropriate for cellular samples.



Select the compensation setup and click **Edit**. Make changes, click **Save**, and close the window.

Recording Specimen Data

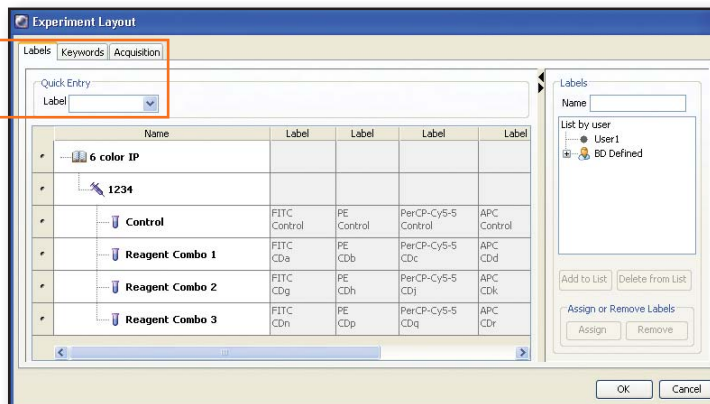
- 1 Create a new experiment by importing the SPA II worklist.
- 2 Link to appropriate cytometer settings.



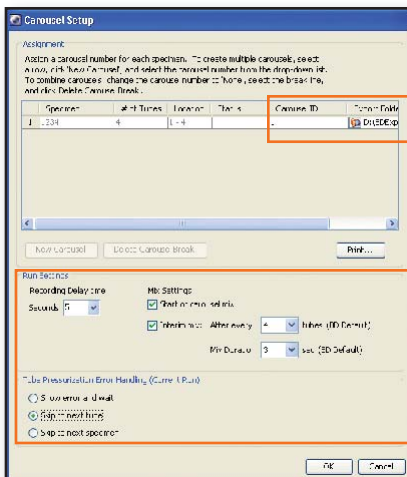
Right-click Cytometer Settings and select Link Setup.

- 3 Verify that selections and entries in the Experiment Layout are appropriate.

Verify parameter labels, keywords, and acquisition criteria are appropriate.

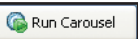


- 4 Verify Carousel Setup settings.



Verify Carousel ID.

Verify Run Settings and Tube Pressurization Error Handling settings.

- 5 Install the carousel on the Loader and click .
- 6 View the Carousel Report and check for any error messages.

Analyzing Data

- 1 Verify that plots, gates, and statistics displayed in worksheets are appropriate for analysis of populations of interest.

Verify gates are set appropriately.

Verify appropriate statistics are displayed.

Use the population hierarchy to verify parent/child relationships.

Population	#Events	%Parent	Mean	Mean	Mean
P1	6,837	33.2	62.18	3,956	9,405
G1	1,847	24.8	212	7,203	38,887
G2	79	1.2	3,859	17,115	18,190
G3	133	1.9	166	5,287	40
G4	4,027	60.7	8,402	1,817	62
G1-1	1,050	16.8	826	23,852	4,814
G2-1	4	0.1	4,873	29,871	18,240
G3-1	607	10.0	534	114	239
G4-1	4,919	74.1	9,095	92	11,654
G1-2	606	9.1	287	20,204	8,317
G2-2	1,076	16.2	428	188	83,088
G3-2	1,112	16.8	750	11,581	57
G4-2	3,844	57.9	8,827	100	84
G1-3	840	12.7	235	18,113	5,775
G2-3	86	0.7	1,722	786	30,664
G3-3	876	13.2	928	10,228	239
G4-3	4,877	73.0	8,879	132	11,419

- 2 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 documents.
 - 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 PDF and exported statistics files.

- 3 Review printouts and verify that the analysis is appropriate.

Shutting Down the System

- 1 Verify that the flow rate in the **Acquisition Dashboard** is set to **Medium** or **High**.
- 2 Select **Carousel > Clean**.

Select cleaning tubes and time.

Tubes	Time (min)
<input checked="" type="checkbox"/> Cleaning	5
<input checked="" type="checkbox"/> Inset 1	5
<input checked="" type="checkbox"/> Inset 2	5

- 3 Install the carousel with the appropriate cleaning tubes and perform the cleaning cycle.
- 4 Perform a fluidics shutdown.
- 5 Empty waste and refill fluids if prompted to do so.
- 6 Turn off the cytometer main power and shut down the computer.