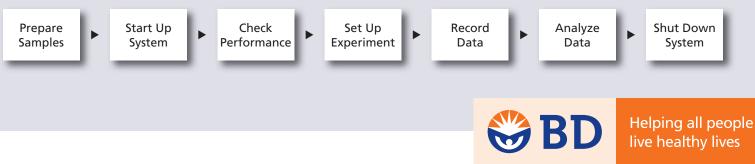
BD FACSDiva Software Quick Reference Guide for **BD FACSCanto Systems with Loader Option**

This guide contains instructions for using BD FACSDiva[™] software version 6 with BD FACSCanto[™] and BD FACSCanto II systems equipped with the BD FACS[™] Loader option. The workflow shown uses the BD FACS[™] Sample Prep Assistant III (SPA III) and BD FACS[™] 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.



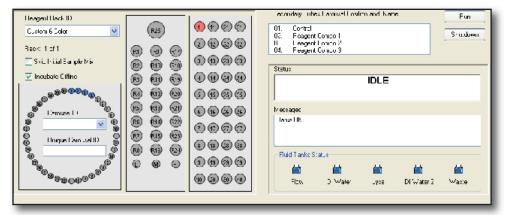
Preparing Samples

Perform daily inspection and startup for the SPA III and startup for the LWA.

2 Set up the worklist in the BD FACS[™] SPA software.

🛊 BD	FACS SPA						56
10 :	otup Enstrument /	Administration Hold					
	Worklis:						
	Sampeikame	Sample 10	Case Number	Frimary Tube Type	Frimary Reck Position	Eare Name	Caro_sel Postion
	osupervale						Calobeer ou der

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



- 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.
- Perform daily cleaning for the SPA III and LWA.
- 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.
- 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	

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

Select Cytometer > CST.

le Gytameter Iaals		
Reports Performance Tracking		
System Summary: DK	Setus Central - Reased User Only - 49 Load a connexite Hith the book Jakes and click Run button to clart Check Performance.	
Cytometer Configuration: 2-Nexer, 6-color (4-2) (BD default) Lot (D: 663+1	East Tube Hancely	Clear this checkbo
Cytometer Baseline: May 23, 2007 12:27 PM	Cytometer Configuration 2-later, 6-color (+-2) (80 default) Select Configuration	Verify the Current
Cytometer Performance: 34y 13, 2007 02:49 PM Cytometer Performance: Result: Passed Cytometer Performance Result: Passed	Setup Brads Lot ID: 66341	Configuration and bead Lot ID.
	Pitoduct CST Setup Beads Plan # 663391 Expendion Date: 10-10-2007	

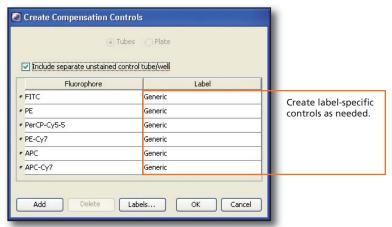
- 2 Place a tube of the BD[™] Cytometer Setup and Tracking beads* in position 1 on a carousel and run the beads.
- 3 View the Cytometer Performance Report.
- **4** Close the **Cytometer Setup and Tracking** window.

Setting Up the Experiment

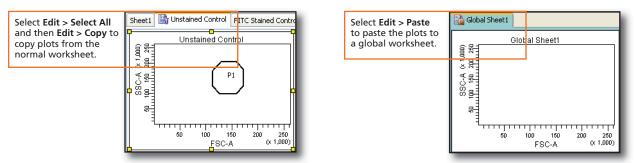
- Select Edit > User Preferences and verify that selected preferences are appropriate.
- 2 Create an experiment in the Browser.
- 8 Right-click Settings in the Browser. Select Application Settings > Apply.

	Application Settings				Cytometer Settings Mismatch	
	Cytometer Configura	ation: 2-laser, 7-	color (5-2) (BD default) 🛛 👻	L	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.	
Select an application setting.	Name A Application A Application B	Owner UserName1 UserName1	Date Created 07/16/07 11:39:58 AM 07/16/07 11:40:06 AM Apply Cancel		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.

Select Experiment > Compensation Setup > Create Compensation Controls.



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



6 Specify Carousel Setup settings.

Specify a carousel
>
Print
Print
Print

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

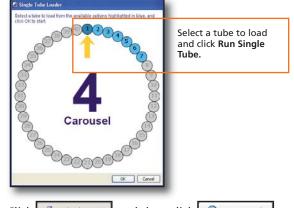
If using compensation beads, write down the FSC, SSC, and threshold values displayed in the **Cytometer** window.

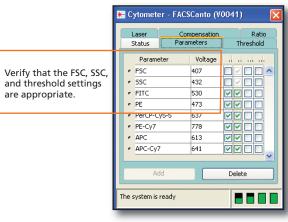
Place compensation control tubes in the carousel in the same order listed in the Browser, and install the carousel in the Loader.

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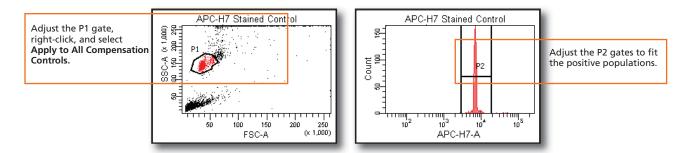
• Verify that the cytometer is configured for automatic loading and that settings are appropriate for the compensation controls.

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





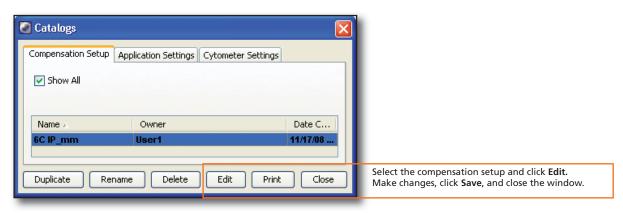
- 1 Click Gunload and then click GRun Carousel.
- View the Carousel Report and check for any error messages.
- ¹³ View recorded data in the normal worksheets and gate the positive populations.



Select Experiment > Compensation Setup > Calculate Compensation.

	Single Stained Setup					
	Compensation calculation has completed successfully					
Rename the compensation setup.	Name: 6 color IP					
	Link & Save Apply Only Cancel					

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



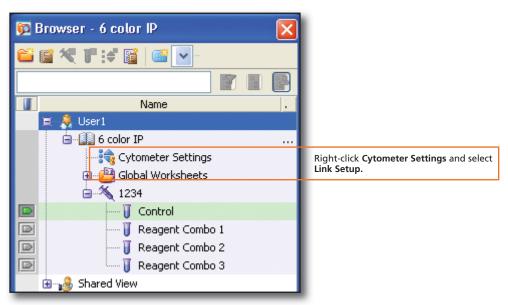
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Recording Specimen Data

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1 Create a new experiment by importing the SPA II worklist.

Link to appropriate cytometer settings.

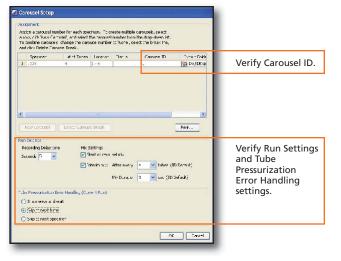


3 Verify that selections and entries in the **Experiment Layout** are appropriate.

	C Experiment Layout									
erify parameter labels,	Labels	Keywords Acquisition								
eywords, and acquisition riteria are appropriate.		ick Entry				j	Labels Name			
		Name	Label Lab		Label	Label	List by user User1			
	•	🕼 6 color IP					🗑 - 🚴 BD Defined			
	•	1234								
	•		FITC Control	PE Control	PerCP-Cy5-5 Control	APC Control				
	•	🛛 🥛 Reagent Combo 1	FITC CDa	PE CDb	PerCP-Cy5-5 CDc	APC CDd				
	•	🔤 🥡 Reagent Combo 2	FITC CDg	PE CDh	PerCP-Cy5-5 CDj	APC CDk	Add to List Delete from List			
		🛛 🗍 Reagent Combo 3	FITC CDn	PE CDp	PerCP-Cy5-5 CDq	APC CDr	Assign or Remove Labels			
		<		-		>				

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• Verify Carousel Setup settings.



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

Analyzing Data

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

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	1234-Reagent off off off off off off off off off off	P1	T. Secondary	1234-Reagen	t Combo 1 Q2 Q1 Q1 Q1 Q1 Q1 Q1 Q1 Q1 Q1 Q1 Q1 Q1 Q1		234-Reagent Combo 1 01-1 02-1 02-1 02-1 02-1 02-1 02-1 02-1 02-1		Verify gates are set appropriately.
Verify appropriate statistics are displayed.	Experiment Hume: Section Mane: Tube Name: Record Date: 01 02 02 02 02 04 04 04 04 04 04 04 04 04 04 04 04 04	#Events 6,607 1,647 79 004 4,067 4,076 4,076 4,076 4,076 4,076 4,076 4,076 4,076 4,076 4,077 4,076 4,077	11 R A 332 248 12 12 100 741 00 741 00 741 100 741 100 741 100 741 100 741 00 741 00 741 00 70 07 07 07	color IP 204 204 205 2008 2:553 CDa FITC-A 62,210 2:12 3:853 985 0:402 8:26 4.573 4.573 2:97 428 7:50 0.927 2:35 4.772	C05 PE-A CD. Mean 3,066 7,203 17,115 5,367 1,917 23,852 25,071 114 92 20,204 596 11,581 90 10,113 765	Maan 9,405 38,867 18,150 40 62 4,614 10,243 309 11,654 0,317 63,089 57,055 30,654	Tube: Respect Combo 1 Providion All Brenk C 01 C 02 C 0		Use the population hierarchy to verify parent/child relationships.
	23-3 ≥ 04-3	875 4,877	13.2 73.5	925 6,879	10,226 132	239 11,479		~	

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).

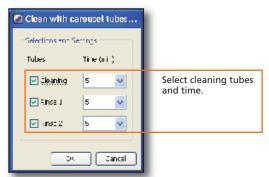
	Batch Analysis			
	 Auto View Time: 10 ▼ Manual 		; iexponential Scales erred Global Worksheet	Select to print, save as a PDF, or export the statistics as needed.
Specify where to save PDF and exported statistics files.		t\Batch_Analysis_05072007133515.pdf s\Batch_Analysis_05072007133515.csv	Browse View PDF Browse	
	Status: 0% Start Pause		Continue Close	

3 Review printouts and verify that the analysis is appropriate.

Shutting Down the System

• Verify that the flow rate in the Acquisition Dashboard is set to Medium or High.

2 Select Carousel > Clean.



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

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- 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.