## BD FACSDiva Software Quick Reference Guide for the BD LSR II or BD LSRFortessa

This guide contains instructions for using BD FACSDiva<sup>™</sup> software version 8.0 and later with BD<sup>™</sup> LSR II or BD LSRFortessa<sup>™</sup> flow cytometers.

# **Workflow Overview**

The following figure shows the daily flow cytometry workflow when using BD FACSDiva software.



- 2 Start BD FACSDiva software and log in.
- Prepare the fluidics tanks and remove bubbles from the fluidics system.
- Verify that the optical filters are appropriate for your experiment.

### **Checking Cytometer Performance**

**1** Select Cytometer > CST.

	Cytometer Setup and Tracking			_ 8 ×	
	Ele Cytometer Tools				
	Setup Reports Performance Tracking				
			Setup Control	(A)	
	System Summary: OK		Load a plate with the beads and cl	ick Run button to start Check	
Verify the Cytometer Configuration and	Cytometer Configuration: (BDDefault)SORP-HTS Lot ID: 45765		Performance.	Abot	
bead lot ID.			Load Tube Manually		
	Cytometer Baseline: July 10, 2013 10:47 AM Cytometer Performance: December 02, 2013 02:42 P Cytometer Performance Results: Passed	и	Plate Type: 96 Well U Bottom Cytometer Configuration (BDDef Select Co Setup Beads	ault)SORP-HTS	If needed, select a different configuration or bead lot ID.
			Product: CST Setup Be Part #: 910858 Expiration Date: 03-31-2014	eads	
			Status	#	
			Parameter Va	ilue	
			Fluidics	Standby	
			Plate Loader	ox	

- 2 Run the BD FACSDiva™ CS&T research beads.
- **3** View the Cytometer Performance Report.
- Close the Cytometer Setup and Tracking window.

### **Setting Up the Experiment**

1 Create Browser elements.

	📴 Browser - Experiment A		X
Use the Browser toolbar to add elements.	🏜 🗃 餐 🍞 🥩 📓 🔤 💽		
	Name	Date	Size
	🗐 🖳 😓 UserName1		
folder —	🛶 😑 💼 My Experiments		
experiment —	🚍 💷 Experiment A	3/19/12 11	
	Cytometer Settings		
	😑 📴 Global Worksheets		
	Global Sheet 1		

**2** Right-click Cytometer Settings in the Browser. Select Application Settings > Apply.

	Application Settings			Cytometer Settings Mismatch		
	Cytometer Configuration: LSR II		I	The application settings to be applied do not match the selected cytometer settings. The following parameters are not in the selected application settings: PerCP-Cy5-5-A, Alexa Fluor 405-A, Alexa Fluor 430-A, DAPI-A, Indo 1 (Blue)-A.		
Select an application setting.	Name A Application A L Application B L	Owner Jser1 Jser1	Date Created 07/05/07 12:52:24 PM 07/05/07 12:53:28 PM	l	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.	
	View		Apply Cancel		Apply Overwrite Cancel Cl Ov if	iick verwrite necessary.

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

Create Compensation Control	s	
<ul> <li>Tubes</li> <li>Include separate unstained control</li> </ul>	⊖ Plate tube/well	
Fluorophore	Label	
• FITC	Generic	
≠ PE	Generic	Create label-specific
≠ PE-Cy7	Generic	controls as needed.
<ul> <li>APC</li> </ul>	Generic	
• APC-Cy7	Generic	
Add Delete Lab		

Install the unstained control tube onto the cytometer. Click
 Acquire Data

	Ӿ Cytometer - LSRII (1)		📰 Normal Worksheet - Unstained Control	
Verify that the FSC, SSC, and threshold settings are appropriate.	Status Parameters Threshold Parameter • FSC • SSC	Laser         Compensation         Ratio           Voltage          A         H            485            A           251	Sheet Unstained Control	View data in the normal worksheets provided.
	FITC     PE     PE-Cy7     APC     APC     Add  Cytometer Connected	466	(000 000 000 000 000 000 000 000	

S Record data for the compensation control tubes.



6 Select Experiment > Compensation Setup > Calculate Compensation.

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

### **Recording Specimen Data**

- 1 Create Browser elements.
- 2 Create the plots, gates, and statistics needed for recording.



#### 3 Make entries in the Experiment Layout.

	🛿 Experiment Layout				
	Labels Keywords Acquisition				
Specify reagent labels, keywords, and acquisition criteria as needed.	Quick Entry           Events to Record         10,000           Global Worksheet         Storage	Gate Stopping T	<b>*</b>	Events to Record Number List by user User1	
	Name	Events to Rec Global Worksh	Stopping Gate Storage Gate	Stopping Time	🗄 😪 BD Defined
	Compensation Controls	5,000	All Events	0	
	FITC Stained Control	5,000	All Events All Events	0	
	Fe Stained Control	5,000	All Events All Events	0	
	APC Stained Control	5,000	All Events All Events	0	
	APC-Cy7 Stained Control	5,000	All Events All Events	0	
	Sample     Tube 001	10,000 🗸	All Events All Events	0	Add to List Datate from List
	r Tube_002	10,000	All Events All Events	0	
	r ↓ Tube_003	10,000	All Events All Events	0	Assign Events
					Assign
	1				OK Cancel

#### 4 Record data.

### **Analyzing Data**

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



#### 2 Verify the analysis.



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).



### **Shutting Down the System**

- Clean the fluidics.
- 2 Select File > Quit.
- **3** Turn off the cytometer and computer.