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

This guide contains instructions for using BD FACSDiva[™] software version 8.0 and later with BD FACSCanto[™] and BD FACSCanto[™] II systems equipped with the BD[™] High Throughput Sampler (HTS) option.

Most of the features for running plate-based experiments using the HTS option are located in the Plate window. The following figure displays the Setup tab of the Plate window.





Helping all people live healthy lives

Workflow Overview

The following figure shows the daily flow cytometry workflow when using 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 software for your use. This guide shows a workflow that uses application settings.

Starting Up the System

1 Start up the cytometer system.

2 Start BD FACSDiva software and log in.



1

Verify that the HTS doors are closed and perform a fluidics startup.

3

Checking Cytometer Performance

- **1** Select Cytometer > CST. 🜒 Cytometer Setup and Tracking <u>F</u>ile <u>⊆</u>ytometer <u>T</u>ools Setup Reports Performance Tracking Setup Contro Load a plate with the beads and click Run button to start Check Performance. System Summary: OK 🕞 Run 🚺 Abort Verify the Cytometer Cytometer Configuration: Configuration Configuration and Lot ID: 34278 bead Lot ID. Load Tube Manually Clear the checkbox and select the Plate Type. Plate Type: 96 Well U Bottom ~ March 19, 2012 11:07 AM Cytometer Baseline: Cytometer Configuration Configuration Sytometer Performance: March 19, 2012 11:22 AM Select Configuration If needed, select a different configuration or bead lot ID. Cytometer Performance Results: Passed Setup Beads Lot ID: 34278 (RUO)
- 2 Run the BD FACSDiva[™] CS&T research beads.
- **3** View the Cytometer Performance Report.
- **4** Close the Cytometer Setup and Tracking window.

Setting Up the Experiment

1 Create Browser elements.



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



3 Create setup control wells.

	ate - 96 Well - U bottom	3
	pecumen rype Normal Augustation or there Normal Augustation Normal Augustation	Select the throughput mode.
Use the Plate toolbar to add	I 2 3 4 5 6 7 9 10 11 12	
wens to the plate layout.		
	○ ○	

9 Select the first well for the compensation controls, right-click, and select Setup > Create Compensation Controls.

5 Create specimen wells.

🔄 Plate - 96 Well - U bottom 🛛 🔀	
Setup Analysis	
Filter Setup Details	
List of specimens on the plate	
A A C C C C C C C C C C C C C C C C C C	Rename the specimen.
D 3 3 3 3 3 3 3 2 3 2 1 Coder Settings Sample Flow Rate (uL/sec) 1.0 W	
E 22 23 23 23 23 23 23 23 23 23 23 23 23	Verify that the loader settings are
F O O O O O O Moving Volume (µ,4) IOO C I Moving Speed (µ,4/sec) Ioo C I Ioo I Ioo C I	appropriate for your
G ○ ○ ○ ○ ○ ○ ○ ○ ○ ○ ○ ○ ○ ○ □ Number of Mixes 2 ♥	recorded events.
H O O O O O O O O O Wash Volume (µL) 400 C	

6 Create a global worksheet.



Install the prepared plate onto the HTS and close the HTS doors.

8 Select the Setup Control well and click ^{Select} Select Setup Control well and click ^{Select} Setup Control well and click ^{Select} Setup Control well and click ^{Select} Setup Control well Setup Contro



Select all the compensation control wells and click select all the compensation control wells

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



1 Select Experiment > Compensation Setup > Calculate Compensation.



Recording Specimen Data

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

	🔛 Global Worksheet - Global Sheet1	×
Use the Worksheet toolbar to create plots and gates.	■ ● 2 ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ● ●	
	Sample-Tube_001 Sample-Tube_001 0 Sample-Tube_001 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	

2 Select the first specimen well and click

Analyzing Data

Under the Analysis tab of the Plate window, select a recorded well.



2 Create plots, gates, and statistics needed for analysis on a global worksheet.

	😰 Browser - Experiment_001	🖀 Global Worksheet - Global Sheet2 🛛 🔀	
	8 5 % 7 ; ≠ 2 2	◙।ऄॼॎख़ॾॎॿॖॾॴख़ॿ	
		▶ ▶ 圖 悟 引 司 世 母 邦 詔 (罪)罪 -	Create custom text and graphics.
	Name Date	Global Sheet1 📴 Global Sheet2	
		Sample-Tube 001	
	Experiment_001 7/5/07 1:29:25 PM		
Create new global worksheets.	Global Worksheets - Cal Global Sheet1 - Cal Global Sheet2		
	P Inspector - Dot Plot	S Parent	
Customize plots using the Plot Inspector.	Plot Title Labels Acquisition Dot Plot Tube: Priment_001 Sample.Tube_001	50 100 150 200 250 FSC-A (x 1.000)	
	Y Parameter: PE-A	Population #Events %Parent %Total All Events 10,000 #### 100.0	

3 Verify the analysis.



Right-click a specimen and select Batch Analysis.

	Batch Analysis				
	Auto	Output To Printer	Statistics		Coloct the
	View Time: 10	▼ Save as PDF ▼ Save as XML ▼ Add Report	 ✓ Freeze Biexponential ✓ Use Preferred Global ✓ Save Specimen Report 	Scales Worksheet ts	options needed.
Specify where to save the	PDF Filename:	sMG-Batch_Analysis_021220131	151506.pdf Browse	View PDF	
PDF, XML file, and exported statistics files.	XML Filename:	sMG-Batch_Analysis_021220131	151506.xml Browse		
	Export Filename: le	sMG-Batch_Analysis_021220131	151506.csv Browse		
	Status:	0%	6		
		Start Par	use Continue	Close	

Shutting Down the System

- Create a new experiment in the Browser.
- 2 Select HTS > Clean.



- Install the prepared plate and click OK to begin cleaning.
- Perform a fluidics shutdown.
- **5** Turn off the cytometer, HTS, and computer.

HTS loader settings are specified under the Setup tab of the Plate window. Ensure that the loader settings are appropriate for your sample volume, sample concentration, and the specified events to record.

Default Loader Settings

-Loader Settings		Loader Settings		Loader Settings		Loader Settings	
Sample Flow Rate (µL/sec)	1.0 🗸	Sample Flow Rate (µL/sec)	1.0 🗸	Sample Flow Rate (µL/sec)	0.5 🗸	Sample Flow Rate (µL/sec)	1.0 💌
Sample Volume (µL)	10	Sample Volume (µL)	3 📮 🕇	Sample Volume (µL)	200 📮 🕇	Sample Volume (µL)	10
Mixing Volume (µL)	100 📑	Mixing Volume (µL)	50 📮 🕇	Mixing Volume (µL)	100	Mixing Volume (µL)	100
Mixing Speed (µL/sec)	180 📑	Mixing Speed (µL/sec)	200	Mixing Speed (µL/sec)	180	Mixing Speed (µL/sec)	180
Number of Mixes	2 🗸	Number of Mixes	2 🗸	Number of Mixes	2 🗸	Number of Mixes	2 🗸
Wash Volume (µL)	400 📮	Wash Volume (µL)	200	Wash Volume (µL)	400 🌗	Wash Volume (µL)	400 ‡
Specimen wells using		Specimen wells using	ı High	Setup Control wells		Compensation Contr	ol wells

Standard Throughput mode

Throughput mode

Loader Setting	Description	Important Considerations
Sample Flow Rate	Amount of sample (in μL per second) that is delivered to the flow cell. Select a rate between 0.5 and 3.0 in increments of 0.5 μL per second.	The larger the value entered, the shorter the plate running time, but this increases the sample core, causing more variation of data.
Sample Volume	Amount of sample (in μL) aspirated from the well and delivered to the flow cell. Select a volume between 2 and 200 μL.	For High Throughput mode, the system aspirates a set amount of 22 µL of sample, but records data for a volume between 2 and 10 µL. For Standard Throughput mode, the system aspirates the sample volume amount plus 20 µL. This value does not include the plate-dependent dead volume. Larger volumes can increase run time.
Mixing Volume	Amount of sample (in μ L) aspirated and dispensed from the well to resuspend the particles.	To avoid introducing bubbles into the fluidics, this value should be half the total well volume.
Mixing Speed	Rate (in µL per second) that the mixing volume sample is aspirated and dispensed.	The faster the rate, the more likely that cell shearing occurs, especially for delicate cells. A faster rate can introduce bubbles in the sample delivered to the cytometer and compromise the separator bubble.
Number of Mixes	The number of times the mixing volume sample is aspirated and dispensed at the mixing speed. Select a number between 0 and 5 mixes.	The larger the number, the longer the plate running time.
Wash Volume	Amount of sheath fluid (in μ L) drawn through the HTS fluidics between wells. Select a volume between 200 and 800 μ L.	Enter a higher value to reduce cross contamination between wells. Enter a lower value to decrease the plate running time.
Enable BLR/ BLR Period For the BD FACSCanto system only; does not apply to the BD FACSCanto II system.	Amount of initial data ignored at the start of data recording. Select a value between 5 and 150. The value selected is multiplied by 10 to determine the recording delay in ms.	Enable this feature when you expect a large fluorescence intensity difference between one well and the next. This function delays recording for the selected amount of time multiplied by 10 (in millisec- onds). For example, setting the BLR period to 5 delays recording for the first 50 ms.