

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

This guide contains instructions for using BD FACSDiva™ software version 8.0 and later with BD™ LSR II, BD LSRFortessa™, or BD LSRFortessa X-20 flow cytometers equipped with the BD™ High Throughput Sampler (HTS) option.

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

**Plate Setup Details**  
Select details shown on the plate layout.

**Plate Information**  
Designate throughput mode and view plate status.

**Plate Layout**  
Specify well types, create compensation control wells, and apply cytometer settings.

**Loader Settings**  
Specify and customize sample delivery, sample mixing, between-well washing, and acquisition delay.



Helping all people live healthy lives

## Workflow Overview

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

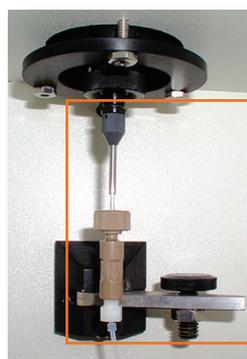


Before starting your daily workflow, ensure that your lab's software administrator has performed all the necessary tasks to set up the software for your use. This guide shows a workflow that uses application settings.

## Starting Up the System

- 1 Start up the cytometer, the computer, and the HTS.
- 2 Prepare the fluidics tanks.
- 3 Verify that the optical filters are appropriate for your experiment.
- 4 Place the cytometer in run mode, start BD FACSDiva software, and log in.

The HTS initializes.

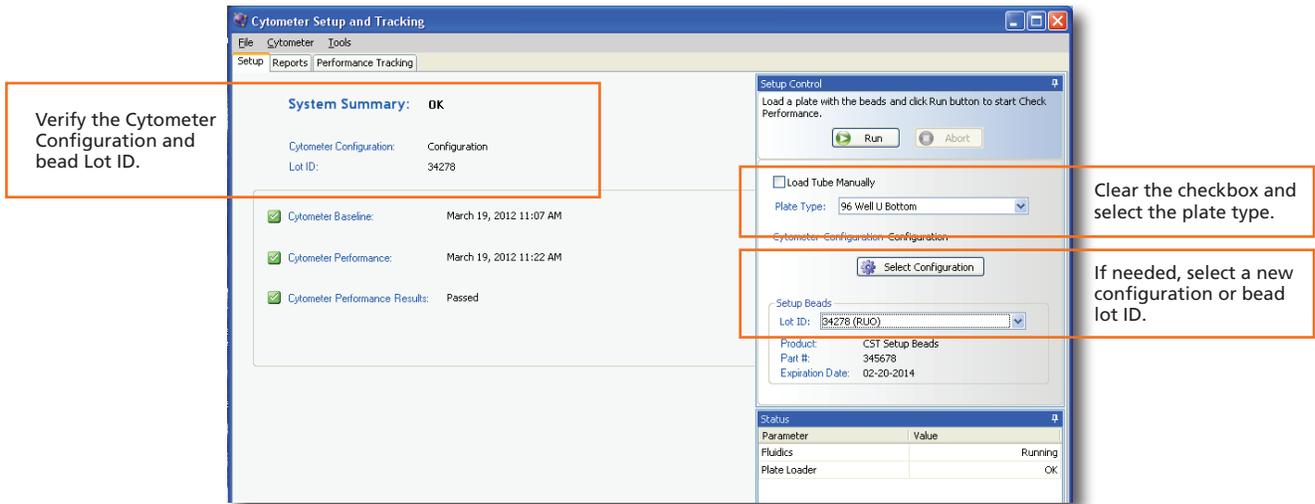


After initialization, verify that the sample coupler is properly installed and not leaking.

- 5 Place the cytometer in standby mode.

## Checking Cytometer Performance

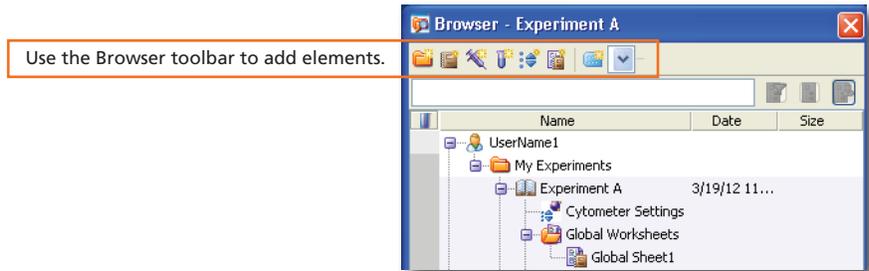
- 1 Select Cytometer > CST.



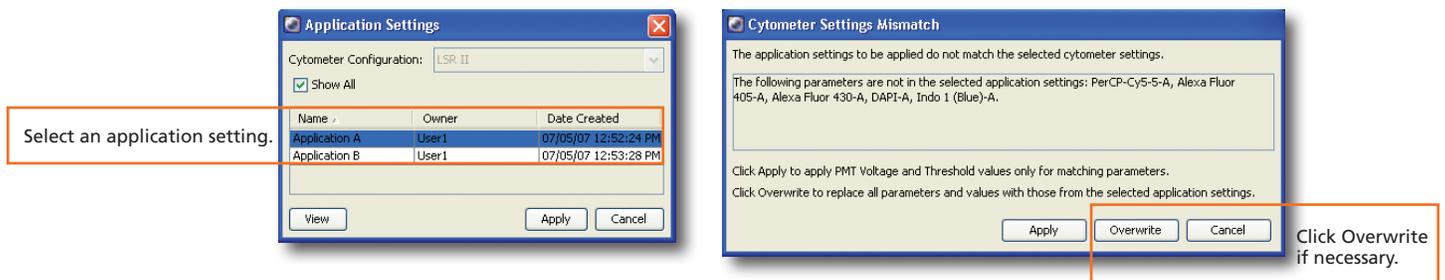
- 2 Place the cytometer in run mode and run the BD FACSDiva™ CS&T research beads.
- 3 View the Cytometer Performance Report.
- 4 Close the Cytometer Setup and Tracking window.
- 5 Place the cytometer in standby mode.

## Setting Up the Experiment

- 1 Create Browser elements.



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



- 3 Create setup control wells.

Use the Plate toolbar to add wells to the plate layout.

Select the throughput mode.

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

- 5 Create specimen wells.

Rename the specimen.

Verify that the loader settings are appropriate for your sample volume.

- 6 Create a global worksheet.

Select all plots and then select Edit > Copy to copy the plots from the normal worksheet.

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

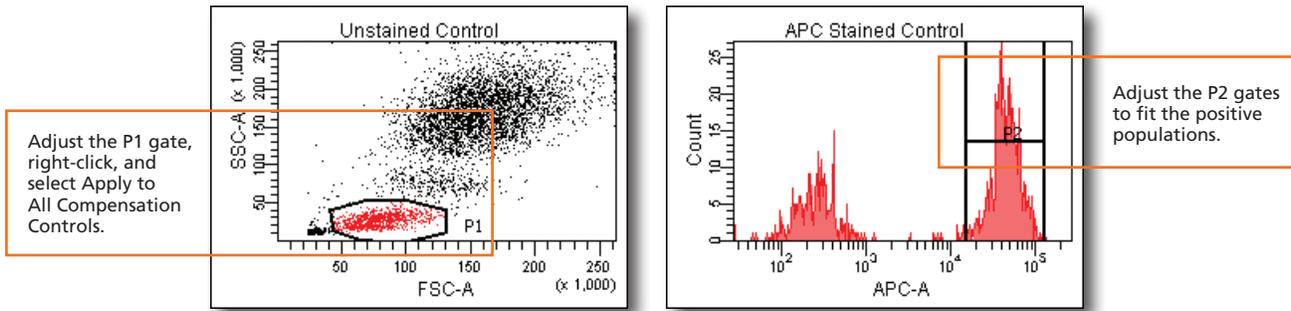
- 7 Install the prepared plate onto the HTS and place the cytometer in run mode.

- 8 Select the Setup Control well and click .

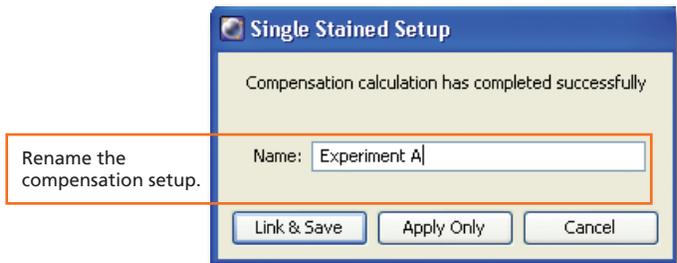
Verify that the FSC, SSC, and threshold settings are appropriate.

Parameter	Voltage	A	H	...
FSC	485	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>
SSC	251	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>
FITC	466	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>
PE	479	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>
PE-Cy7	621	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>
APC	579	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>
APC-Cy7	568	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>

- 9 Select all the compensation control wells and click  .
- 10 View the recorded data in the normal worksheets and gate the positive populations.

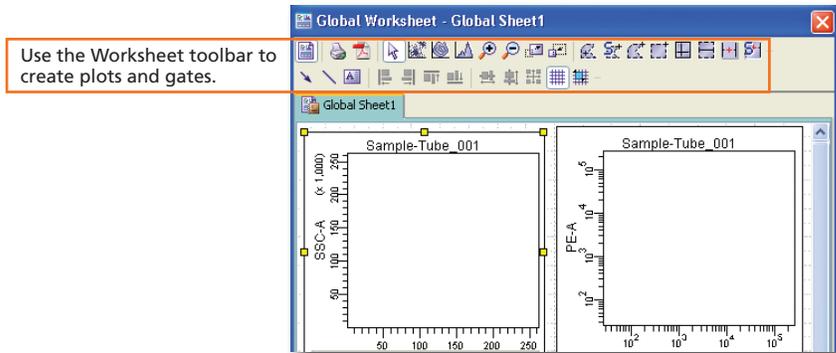


- 11 Select Experiment > Compensation Setup > Calculate Compensation.



## Recording Specimen Data

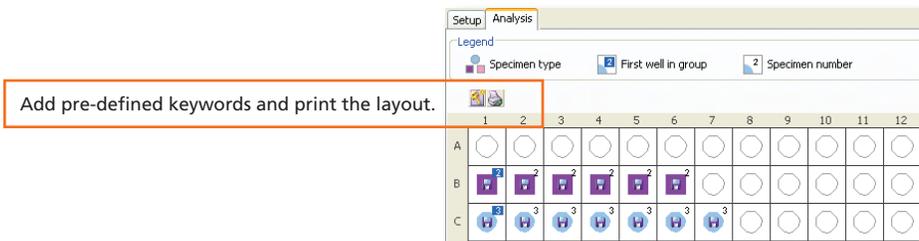
- 1 Create plots, gates, and statistics needed for recording.



- 2 Select the first specimen well and click  .
- 3 When recording is complete, place the cytometer in standby mode.

## Analyzing Data

- 1 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

Inspector - Dot Plot

Create new global worksheets.

Customize plots using the Plot Inspector.

Create custom text and graphics.

- 3 Verify the analysis.

Sample-Tube\_001

Verify that gates are set appropriately for all samples.

Population	#Events	%Parent	%Total
All Events	30,000	###	100.0
Parent	7,966	26.6	26.6
Child A	437	5.5	1.5
Child B	1,645	20.7	5.5

Use the population hierarchy to verify parent/child relationships.

- 4 Right-click a specimen and select Batch Analysis.

Batch Analysis

Auto (selected)

View Time: 10

Manual

Output To Printer (checked)

Statistics (checked)

Save as PDF (checked)

Freeze Biexponential Scales (checked)

Save as XML (checked)

Use Preferred Global Worksheet (checked)

Add Report (checked)

Save Specimen Reports (unchecked)

PDF Filename: lesMG-Batch\_Analysis\_02122013151506.pdf

XML Filename: lesMG-Batch\_Analysis\_02122013151506.xml

Export Filename: lesMG-Batch\_Analysis\_02122013151506.csv

Status: 0%

Select the options needed.

## Shutting Down the System

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

Plate Templates

Clean

Select the Daily Clean template.

Name	Date
Daily Clean - 96 well U-bottom	2/23/06 2:02 PM

Name: Daily Clean - 96 well U-bottom

OK Cancel

- 3 Install the prepared plate and click OK to begin cleaning.
- 4 Select File > Quit.
- 5 Turn off the cytometer and computer.

# HTS Loader Settings Overview

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

Specimen wells using Standard Throughput mode

Specimen wells using High Throughput mode

Setup Control wells

Compensation Control wells

Loader Setting	Description	Important Considerations
Sample Flow Rate	Amount of sample (in $\mu\text{L}$ per second) that is delivered to the flow cell. Select a rate between 0.5 and 3.0 in increments of 0.5 $\mu\text{L}$ 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 $\mu\text{L}$ ) aspirated from the well and delivered to the flow cell. Select a volume between 2 and 200 $\mu\text{L}$ .	This value does not include the system default volume or the plate-dependent dead volume.  For High Throughput mode, the system aspirates a set amount of 22 $\mu\text{L}$ of sample, but records data for a volume between 2 and 10 $\mu\text{L}$ . For Standard Throughput mode, the system aspirates the sample volume amount plus 20 $\mu\text{L}$ .
Mixing Volume	Amount of sample (in $\mu\text{L}$ ) 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 $\mu\text{L}$ 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 $\mu\text{L}$ ) drawn through the HTS fluidics between wells. Select a volume between 200 and 800 $\mu\text{L}$ .	Enter a higher value to reduce cross contamination between wells. Enter a lower value to decrease the plate running time.
Enable BLR/ BLR Period	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. (Feature available on BD LSR II, BD LSRFortessa family, and BD FACSCanto A)  This function delays recording for the selected amount of time multiplied by 10 (in milliseconds). For example, setting the BLR period to 5 delays recording for the first 50 ms.