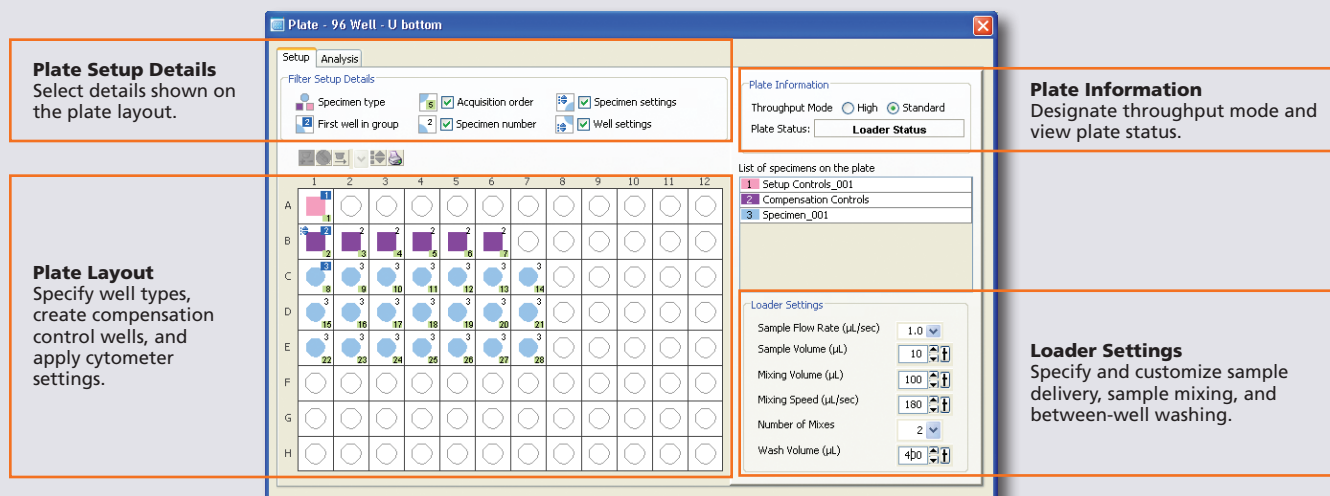


BD FACSDiva Software Quick Reference Guide for the BD LSR II with HTS Option

This guide contains instructions for using BD FACSDiva™ software version 6.0 and later with BD™ LSR II 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.



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Workflow Overview

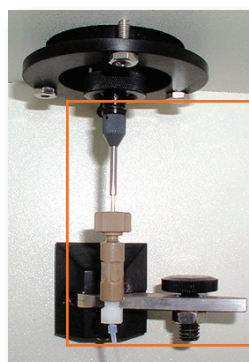
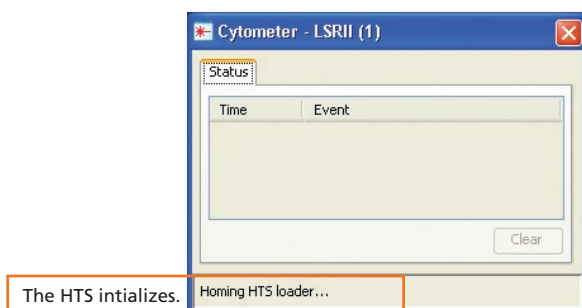
The following figure shows the steps for daily workflow using BD FACSDiva software.



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

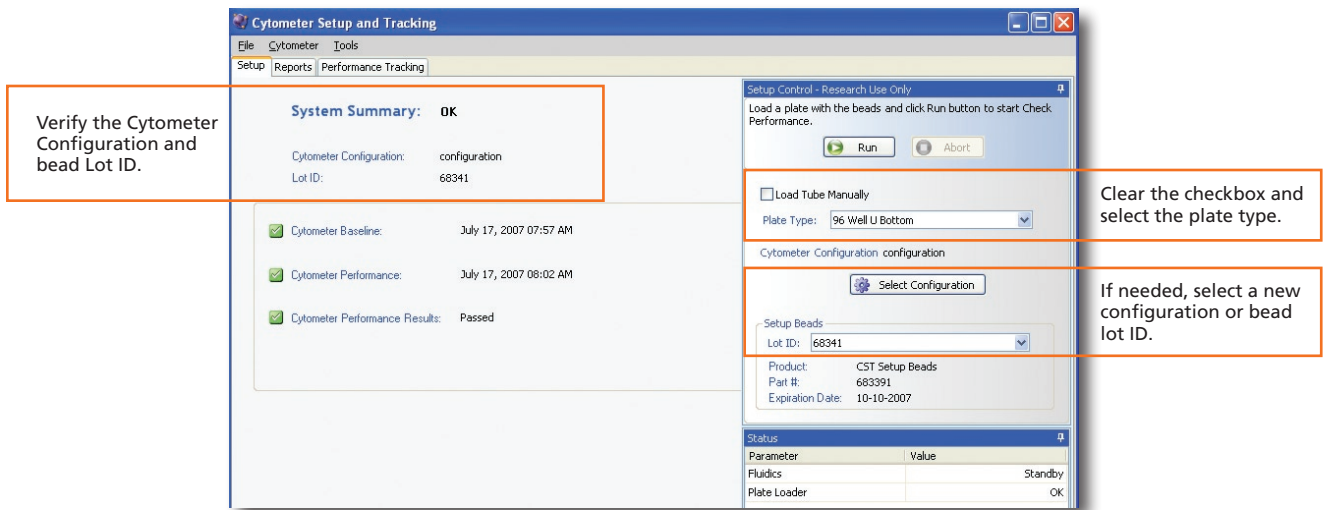
- 1 Start up the cytometer, the computer, and the HTS.
- 2 Prepare the fluidics tanks.
- 3 Verify that the optical filters are appropriate for your experiment.
- 4 Place the cytometer in run mode, start BD FACSDiva software, and log in.



- 5 Place the cytometer in standby mode.

Checking Cytometer Performance

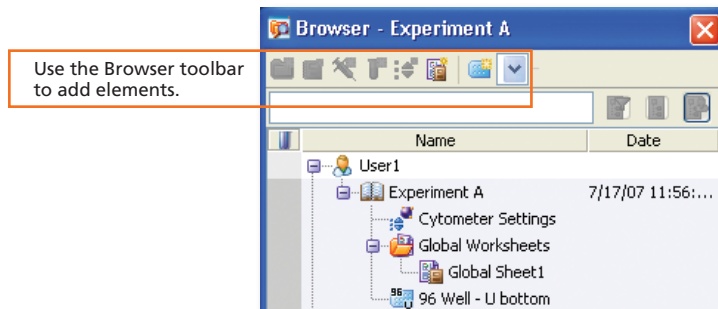
- 1 Select Cytometer > CST.



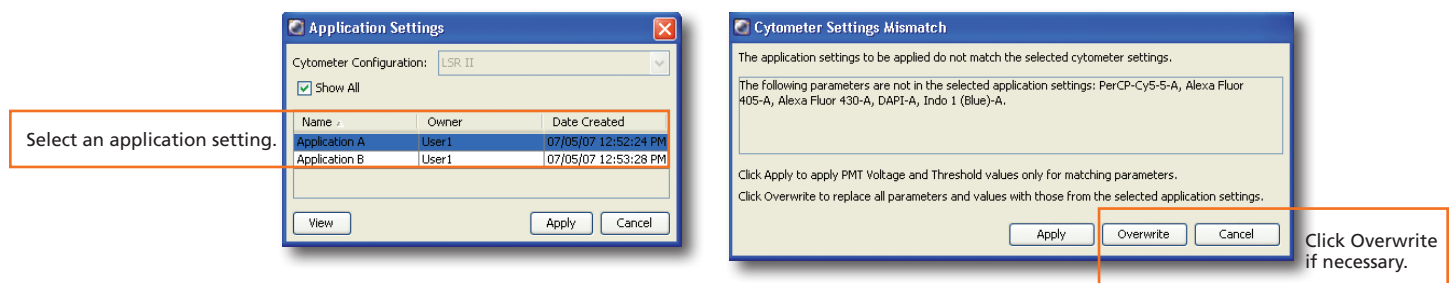
- 2 Place the cytometer in run mode and run the BD™ Cytometer Setup and Tracking beads.
- 3 View the Cytometer Performance Report.
- 4 Close the Cytometer Setup and Tracking window.
- 5 Place the cytometer in standby mode.

Setting Up the Experiment

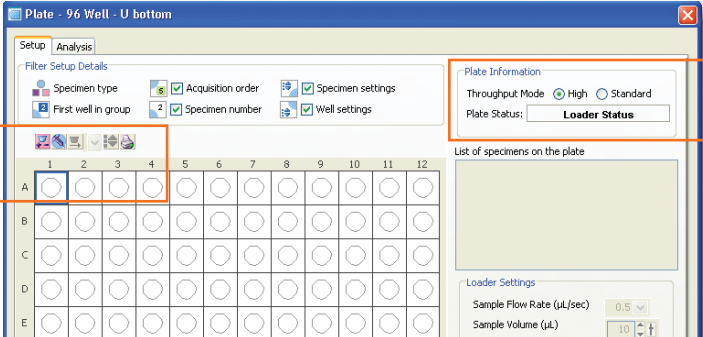
- 1 Create Browser elements.



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



- 3 Create setup control wells.

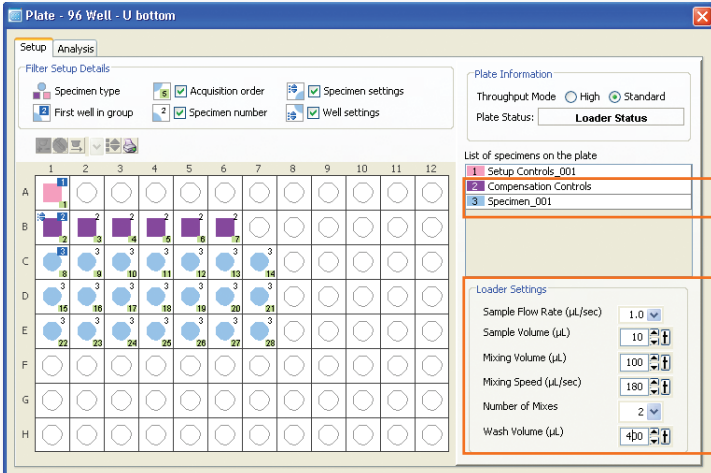


Use the Plate toolbar to add wells to the plate layout.

Select the throughput mode.

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

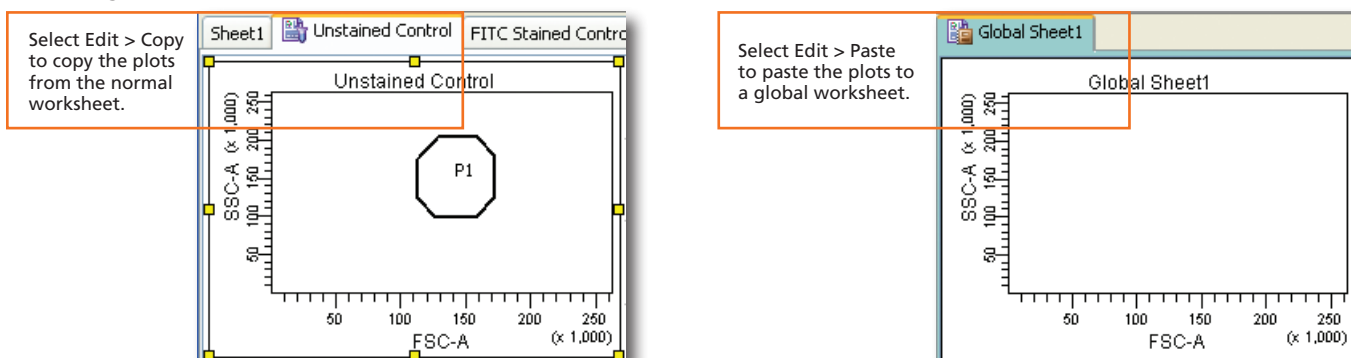
- 5 Create specimen wells.



Rename the specimen.

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

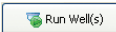
- 6 Create a global worksheet.

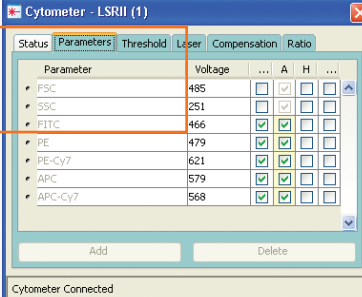


Select Edit > Copy to copy the plots from the normal worksheet.

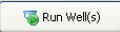
Select Edit > Paste to paste the plots to a global worksheet.

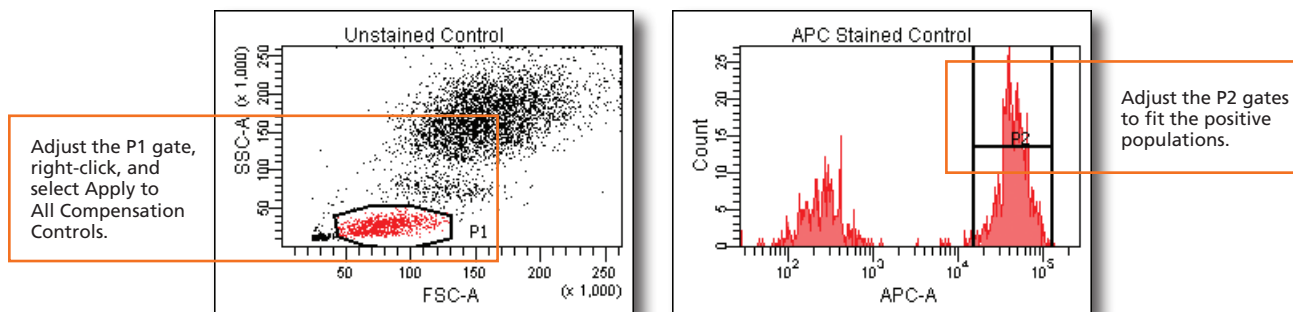
- 7 Install the prepared plate onto the HTS and place the cytometer in run mode.

- 8 Select the Setup Control well and click .

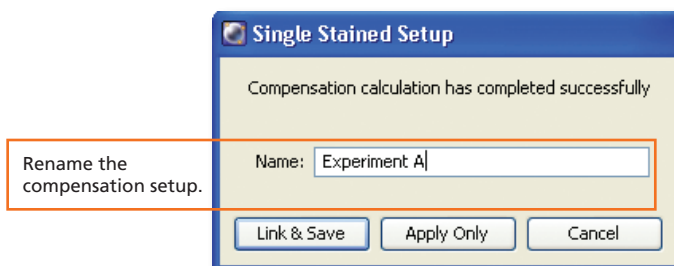


Verify that the FSC, SSC, and threshold settings are appropriate.

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

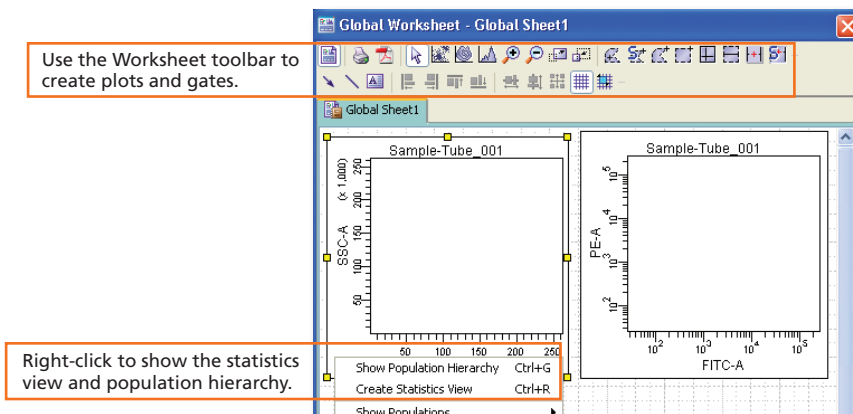


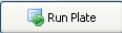
- 11 Select Experiment > Compensation Setup > Calculate Compensation.



Recording Specimen Data

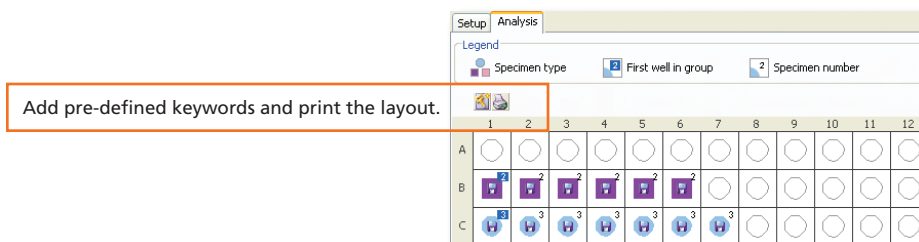
- 1 Create plots, gates, and statistics needed for recording.



- 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

Global Sheet1 Global Sheet2

Sample-Tube_001

SSC-A (x 1,000)

FSC-A (x 1,000)

Parent

Tube: Tube_001

Population	#Events	%Parent	%Total
All Events	10,000	###	100.0

Create custom text and graphics.

Create new global worksheets.

Customize plots using the Plot Inspector.

- 3 Perform quality control of the analysis.

Sample-Tube_001

SSC-A (x 1,000)

FSC-A (x 1,000)

Parent

Tube: Tube_001

Population	#Events	%Parent	%Total
All Events	10,000	###	100.0
Parent	1,867	18.7	18.7
Child A	128	6.9	1.3
Child B	219	11.7	2.2

Verify that gates are set appropriately for all samples.

Use the population hierarchy to verify parent/child relationships.

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

Batch Analysis

Auto [x] Manual []

View Time: 10

Output To Printer [x] Statistics [x]

Save as PDF [x] Freeze Biexponential Scales [x]

Add Report to PDF [x] Use Preferred Global Worksheet [x]

PDF Filename: sheet\Batch_Analysis_05072007133515.pdf Browse... View PDF [x]

Export Filename: itistics\Batch_Analysis_05072007133515.csv Browse...

Status: 0%

Start Pause Continue Close

Select to print, save as a PDF, or export the statistics as needed.

Shutting Down the System

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

Plate Templates

Clean

Name	Date
Daily Clean - 96 well U-bottom	2/28/06 2:02 PM

Name: Daily Clean - 96 well U-bottom

OK Cancel

Select the Daily Clean template.

- 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

Loader Settings

Sample Flow Rate (µL/sec) 1.0

Sample Volume (µL) 10

Mixing Volume (µL) 100

Mixing Speed (µL/sec) 180

Number of Mixes 2

Wash Volume (µL) 400

Loader Settings

Sample Flow Rate (µL/sec) 1.0

Sample Volume (µL) 3

Mixing Volume (µL) 50

Mixing Speed (µL/sec) 200

Number of Mixes 2

Wash Volume (µL) 200

Loader Settings

Sample Flow Rate (µL/sec) 0.5

Sample Volume (µL) 200

Mixing Volume (µL) 100

Mixing Speed (µL/sec) 180

Number of Mixes 2

Wash Volume (µL) 400

Loader Settings

Sample Flow Rate (µL/sec) 1.0

Sample Volume (µL) 10

Mixing Volume (µL) 100

Mixing Speed (µL/sec) 180

Number of Mixes 2

Wash Volume (µL) 400

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 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 system default volume or the plate-dependent dead volume.
Mixing Volume	Amount of sample (in µL) 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 µL) drawn through the HTS fluidics between wells. Select a volume between 200 and 800 µL.	Enter a higher value to reduce cross contamination between wells. Enter a lower value to decrease the plate running time.