

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

This guide contains instructions for using BD FACSDiva™ software version 6.0 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 on the 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, and between-well washing.



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## Workflow Overview

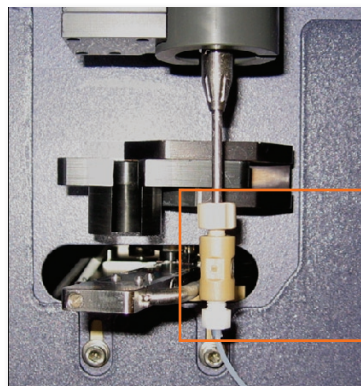
The following figure shows the steps for daily workflow using BD FACSDiva software.



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 Start BD FACSDiva software and log in.



Verify that the sample coupler is properly installed and not leaking.

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

## Checking Cytometer Performance

- 1 Select Cytometer > CST.

Verify the Cytometer Configuration and bead Lot ID.

Clear the checkbox and select the plate type.

If needed, select a different configuration or bead lot ID.

- 2 Run the BD™ Cytometer Setup and Tracking beads.
- 3 View the Cytometer Performance Report.
- 4 Close the Cytometer Setup and Tracking window.

## Setting Up the Experiment

- 1 Create Browser elements.

Use the Browser toolbar to add elements.

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

Select an application setting.

Click Overwrite if necessary.

- 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 > Select All and then Edit > Copy to copy 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 close the HTS doors.

- 8 Select the Setup Control well and click .

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

Parameter	Voltage	...	...	...
FSC	407			
SSC	432			
FITC	530			
PE	473			
PerCP-Cy5-5	637			
PE-Cy7	778			
APC	613			
APC-Cy7	641			

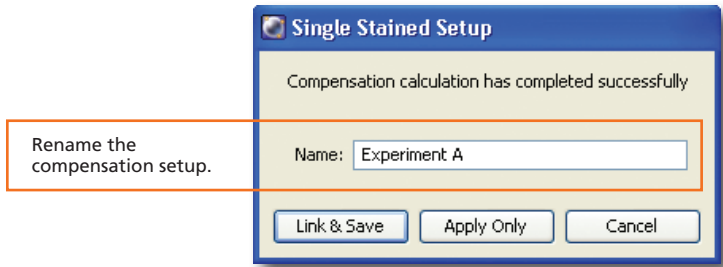
- 9 Select all the compensation control wells and click .

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

Adjust the P1 gate, right-click, and select Apply to All Compensation Controls.

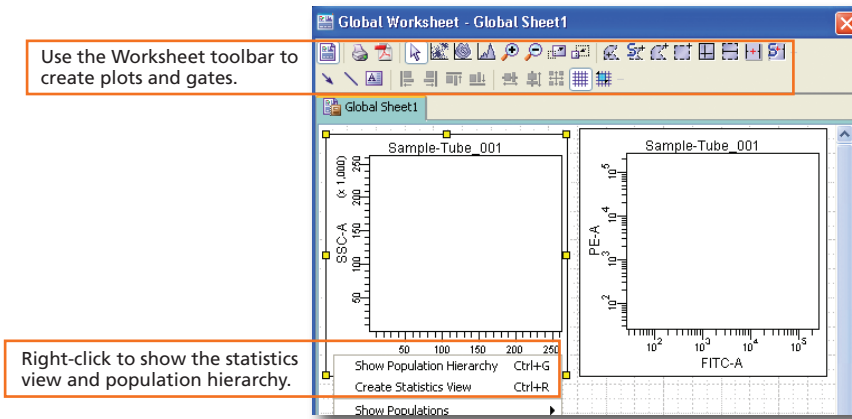
Adjust the P2 gates to fit the positive populations.


- 11 Select Experiment > Compensation Setup > Calculate Compensation.



## Recording Specimen Data

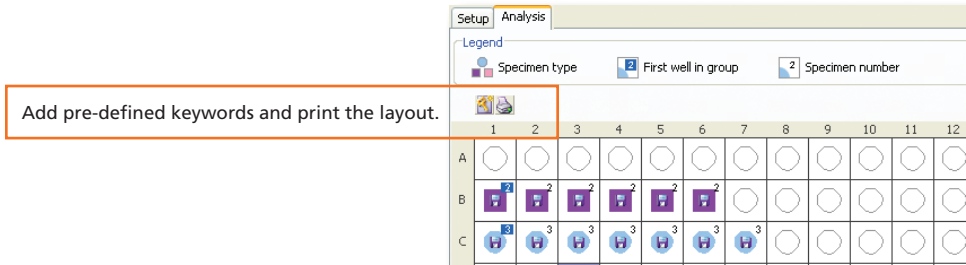
- 1 Create plots, gates, and statistics needed for recording.



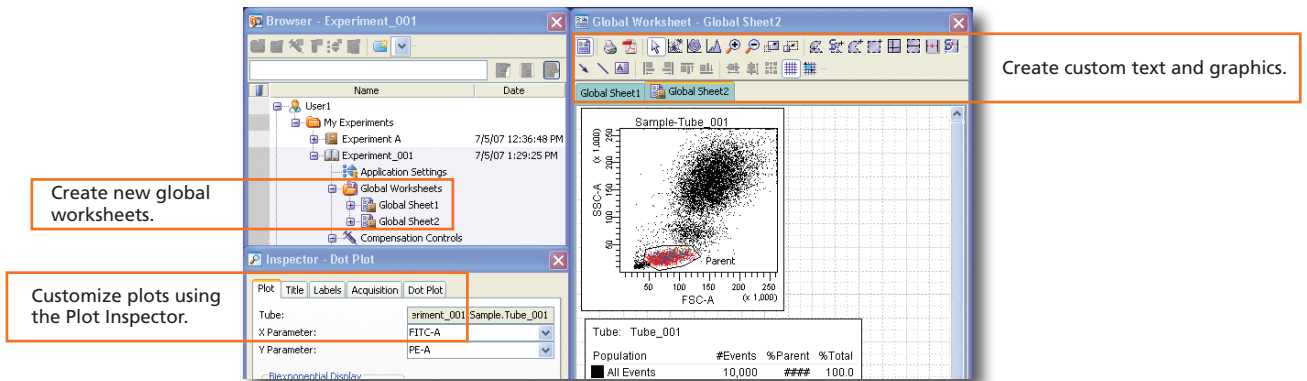
- 2 Select the first specimen well and click  .

## Analyzing Data

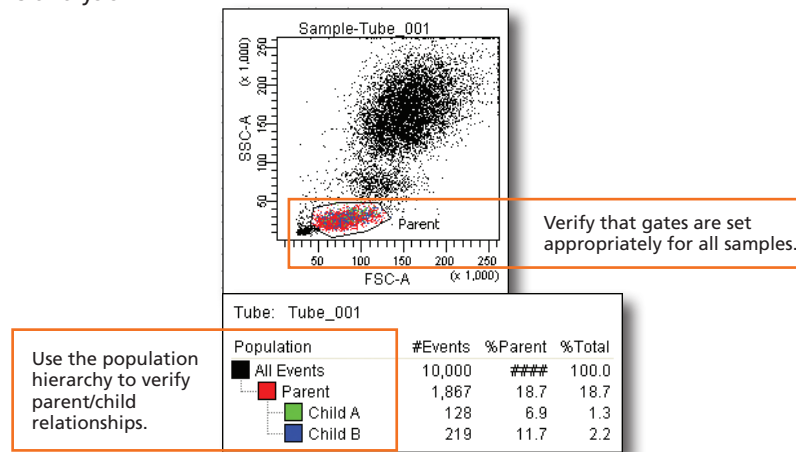
- 1 Under the Analysis tab of the Plate window, select a recorded well.



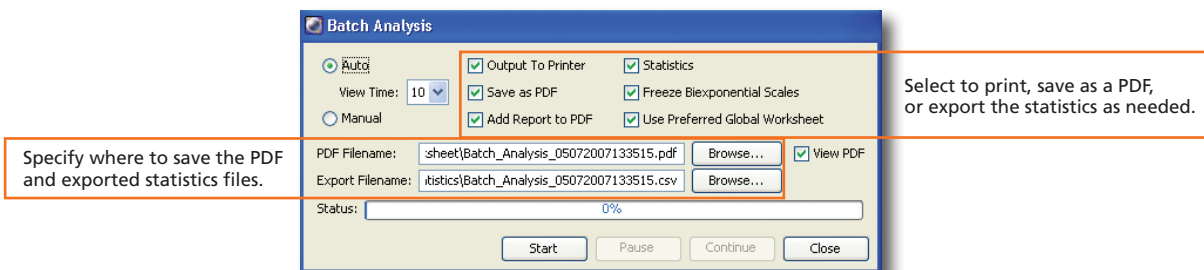
- 2 Create plots, gates, and statistics needed for analysis on a global worksheet.



- 3 Perform quality control of the analysis.

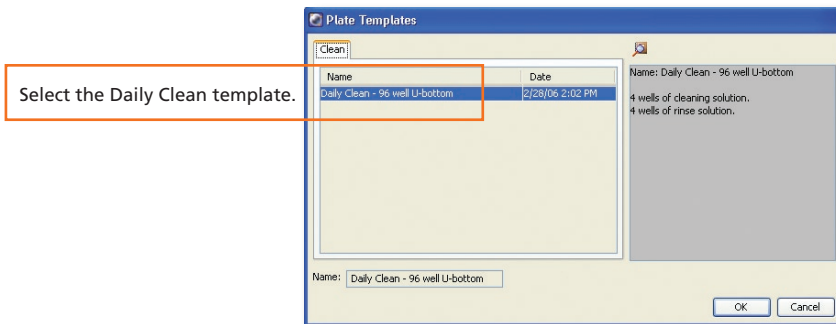


- 4 Right-click a specimen and select Batch Analysis.



## Shutting Down the System

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



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

Well Type	Sample Flow Rate (µL/sec)	Sample Volume (µL)	Mixing Volume (µL)	Mixing Speed (µL/sec)	Number of Mixes	Wash Volume (µL)
Specimen wells using Standard Throughput mode	1.0	10	100	180	2	400
Specimen wells using High Throughput mode	1.0	3	50	200	2	200
Setup Control wells	0.5	200	100	180	2	400
Compensation Control wells	1.0	10	100	180	2	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.

\*BD FACSDiva software version 6.0 is for research use only. Not for therapeutic or diagnostic procedures.