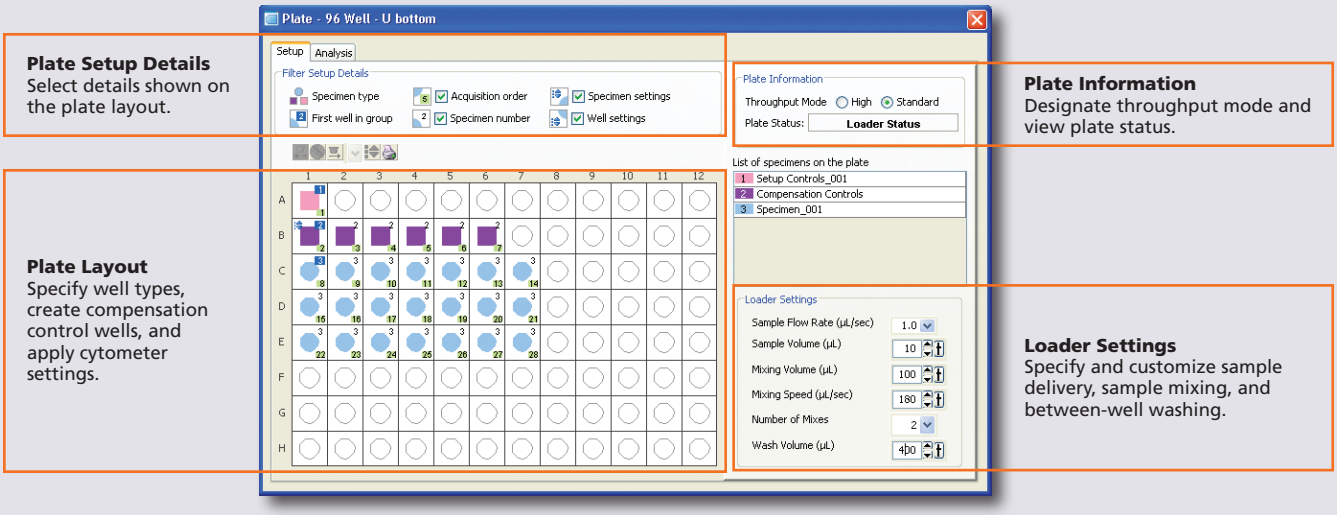
BD FACSDiva Software Quick Reference Guide for BD FACSCanto Systems with HTS Option

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.



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Workflow Overview

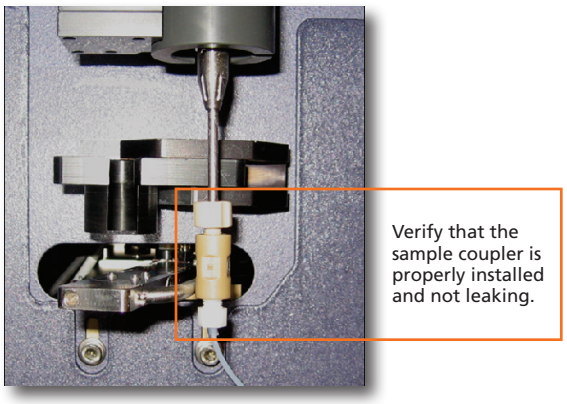
The following figure shows the daily flow cytometry workflow when using BD FACSDiva software.

Start Up System ▶ Check Performance ▶ Set Up Experiment ▶ Record Data ▶ Analyze Data ▶ Shut Down System

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

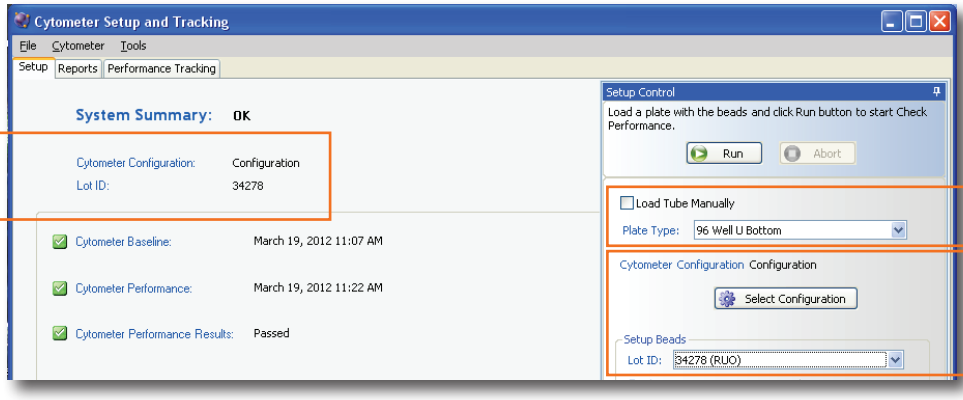
1. Start up the cytometer system.
2. Start BD FACSDiva software and log in.



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.

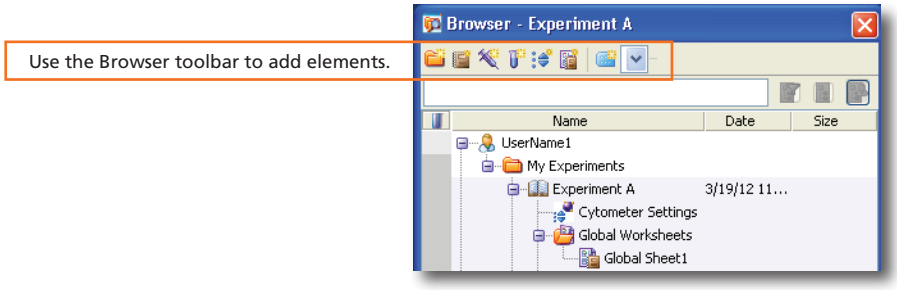
Clear the checkbox and select the Plate Type.

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

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

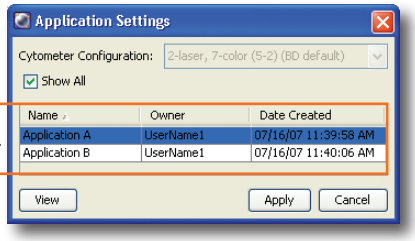
Setting Up the Experiment

1. Create Browser elements.

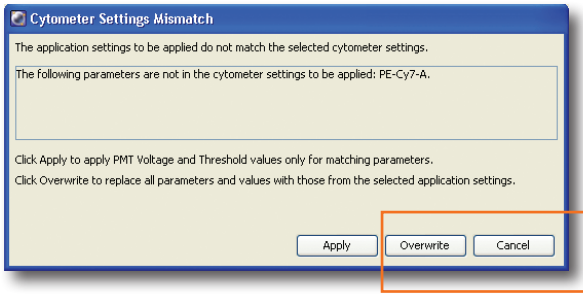


Use the Browser toolbar to add elements.

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

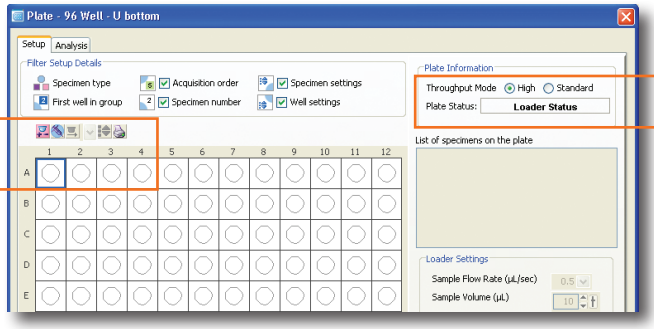


Select an application setting.



Click Overwrite if necessary.

3. Create setup control wells.

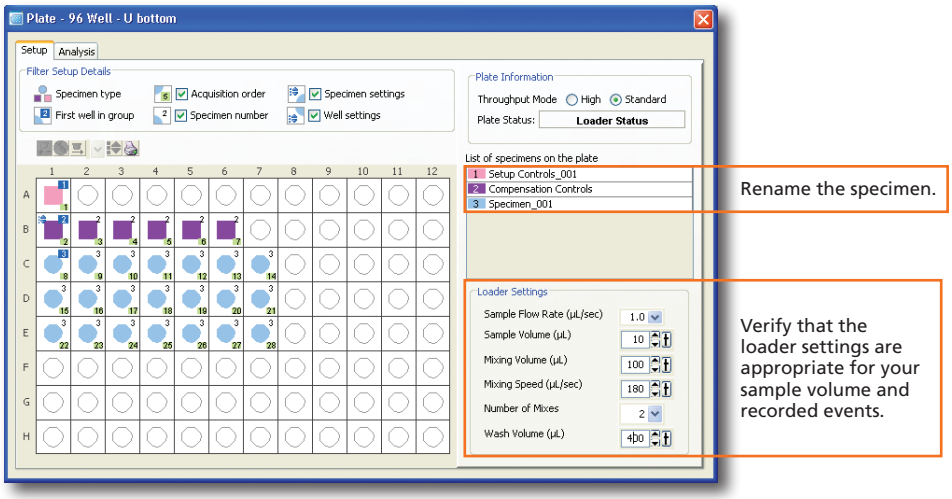


Use the Plate toolbar to add wells to the plate layout.

Select the throughput mode.

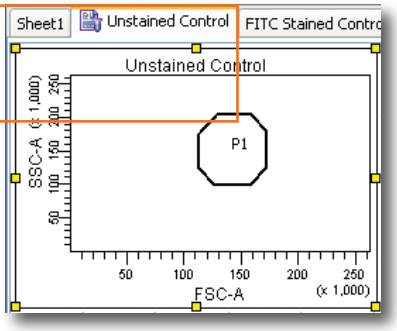
4. Select the first well for the compensation controls, right-click, and select Setup > Create Compensation Controls.

5. Create specimen wells.

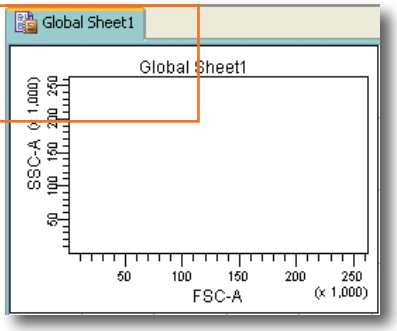


6. Create a global worksheet.

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



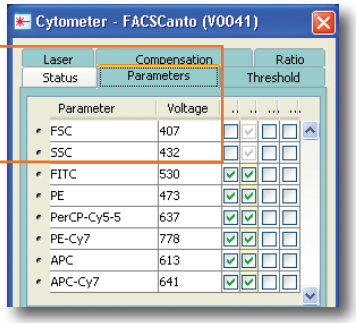
Select Edit > Paste to paste the plots to a global worksheet.



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

8. Select the Setup Control well and click Run Well(s).

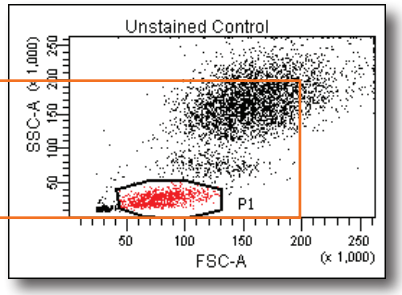
Verify that the FSC, SSC, and threshold settings are appropriate.

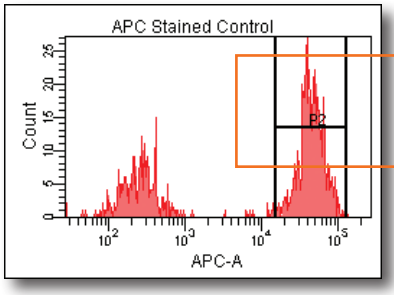


9. Select all the compensation control wells and click Run Well(s).

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

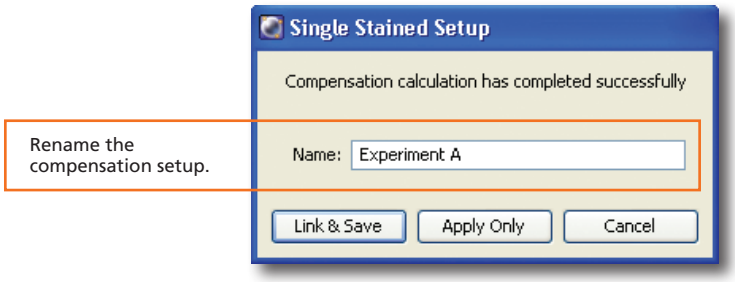
Adjust the P1 gate, right-click, and select Apply to All Compensation Controls.





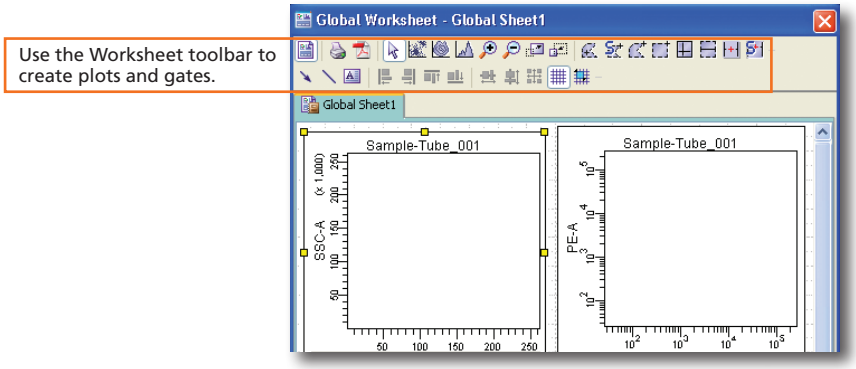
Adjust the P2 gates to fit the positive populations.

11. Select Experiment > Compensation Setup > Calculate Compensation.



Recording Specimen Data

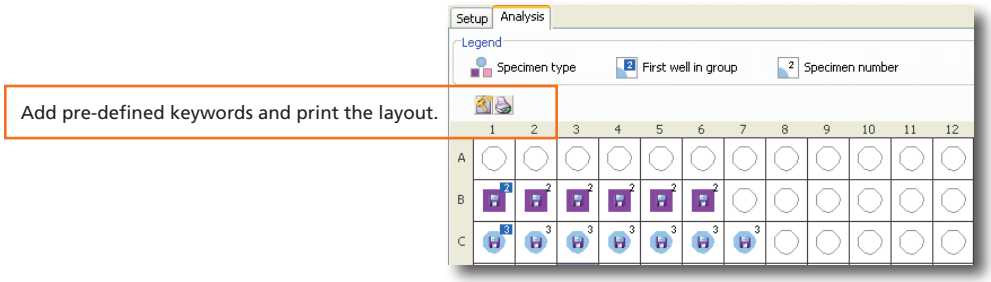
1. Create plots, gates, and statistics needed for recording.



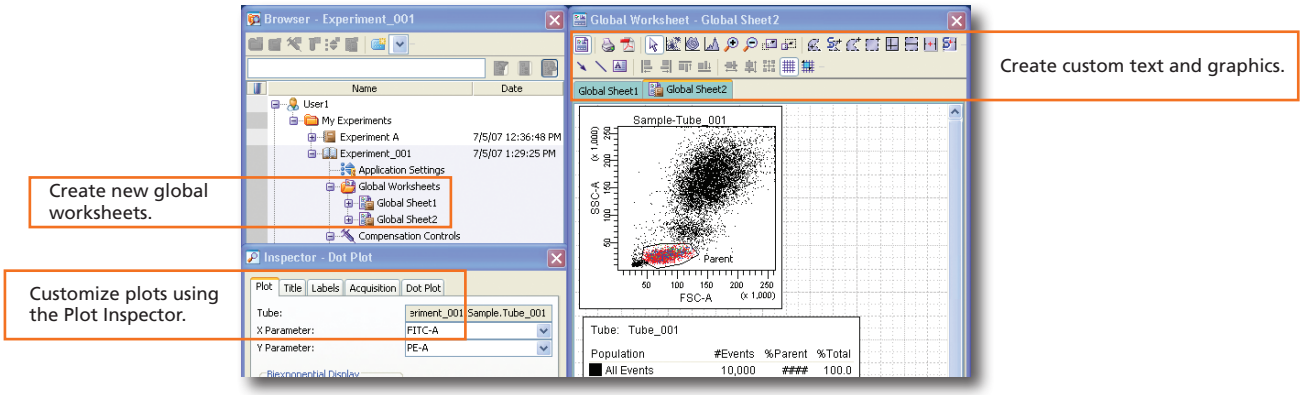
2. Select the first specimen well and click Run Plate .

Analyzing Data

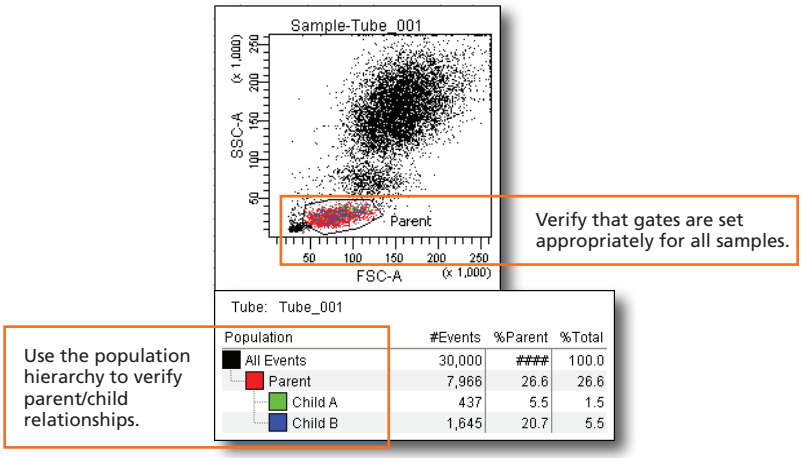
1. Under the Analysis tab of the Plate window, select a recorded well.



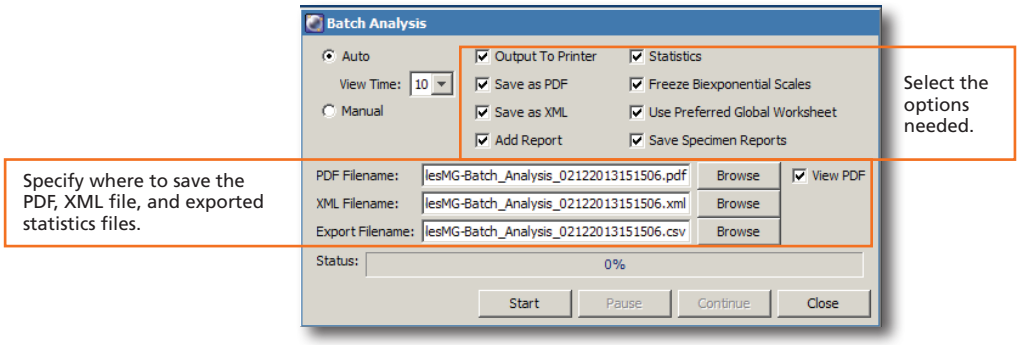
2. Create plots, gates, and statistics needed for analysis on a global worksheet.



3. Verify the analysis.

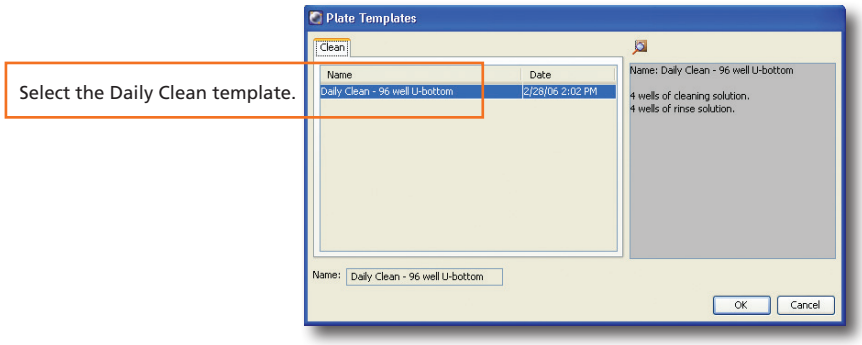


4. Right-click a specimen and select Batch Analysis.



Shutting Down the System

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

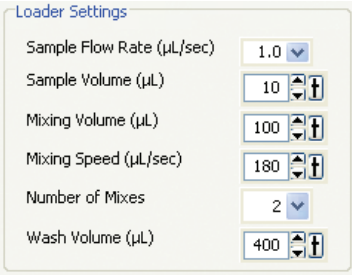


3. Install the prepared plate and click OK to begin cleaning.
4. Perform a fluidics shutdown.
5. Turn off the cytometer, HTS, and computer.

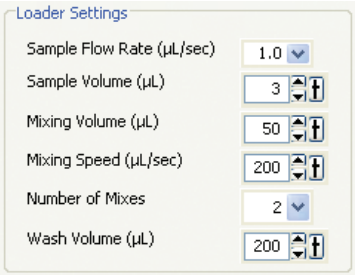
HTS Loader Settings Overview

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.

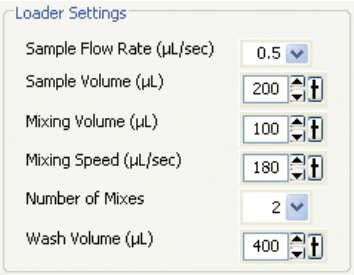
Default Loader Settings



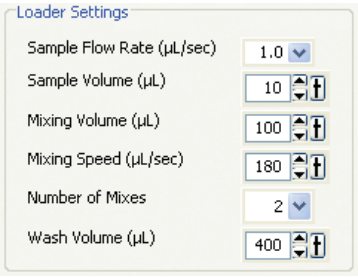
Specimen wells using Standard Throughput mode



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 plate-dependent dead volume. Larger volumes can increase run time.
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.
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 milliseconds). For example, setting the BLR period to 5 delays recording for the first 50 ms.