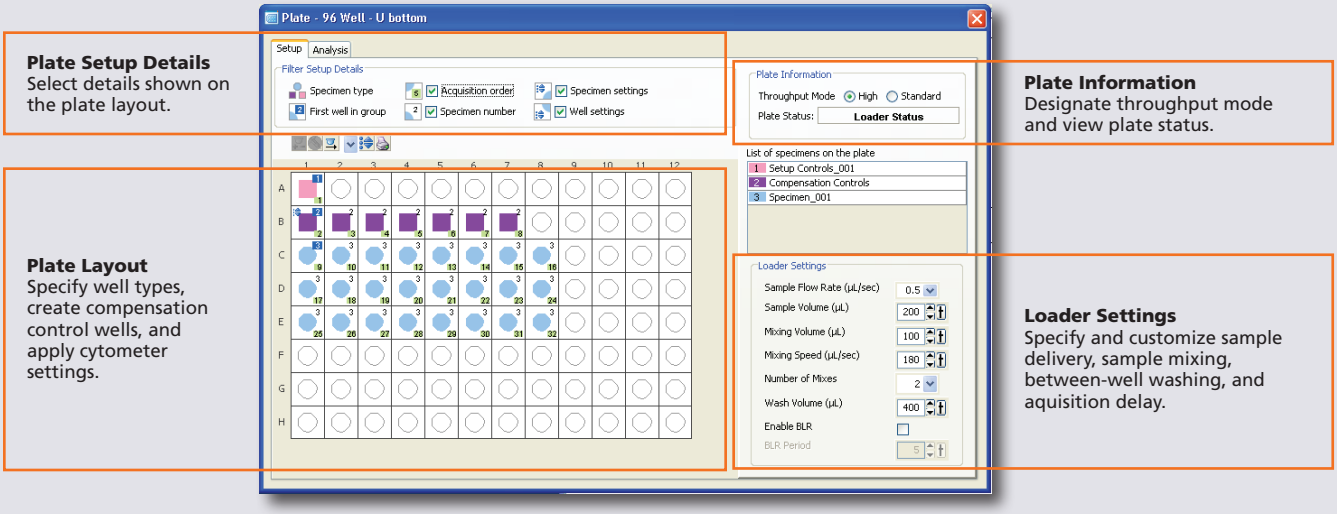
BD FACSDiva Software Quick Reference Guide for the BD LSR II or BD LSRFortessa with HTS Option

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.



BD

Helping all people live healthy lives

Workflow Overview

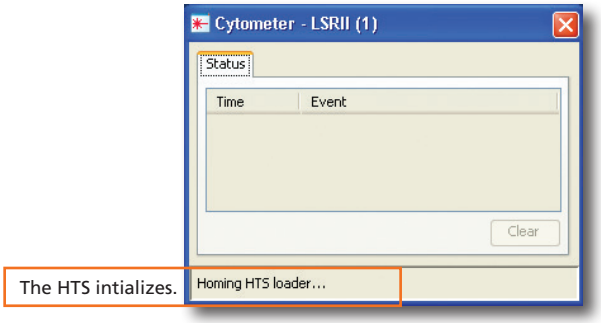
The following figure shows the daily flow cytometry workflow when using BD FACSDiva software.

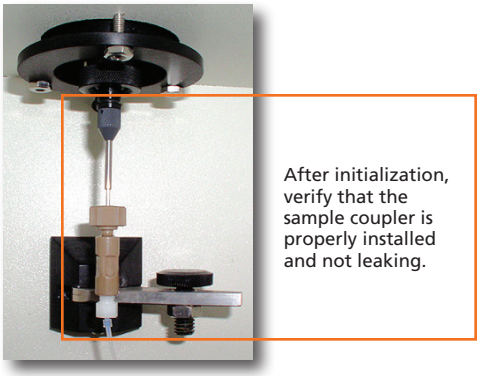
Start Up System ▶ Check Performance ▶ Set Up Experiment ▶ Record Data ▶ Analyze Data ▶ Shut Down System

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, 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.



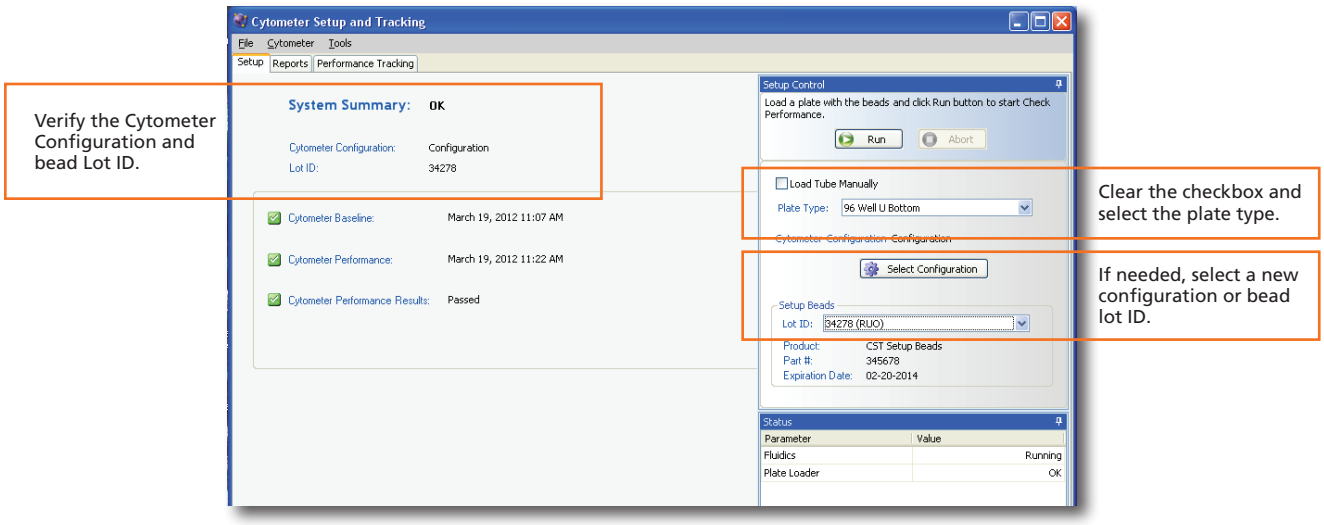


After initialization, verify that the sample coupler is properly installed and not leaking.

5. Place the cytometer in standby mode.

Checking Cytometer Performance

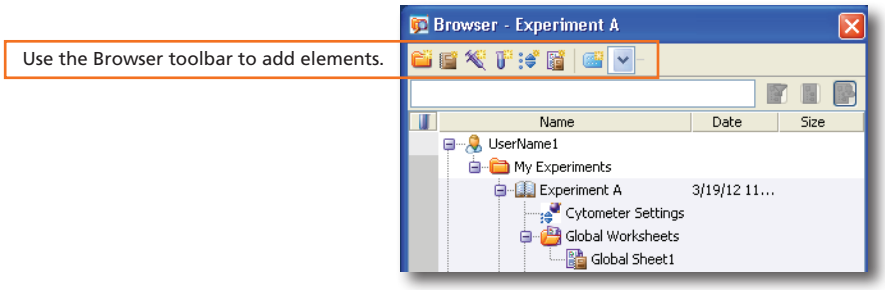
1. Select Cytometer > CST.



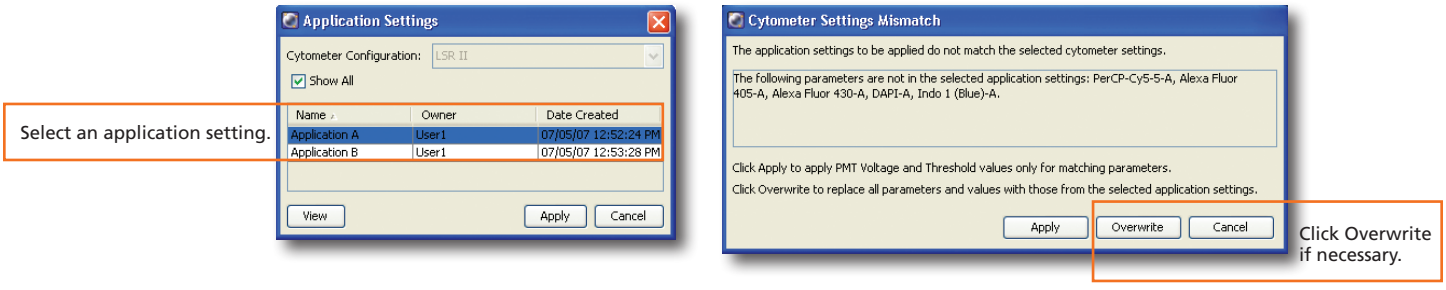
2. Place the cytometer in run mode and run the BD FACSDiva™ CS&T research beads.
3. View the Cytometer Performance Report.
4. Close the Cytometer Setup and Tracking window.
5. Place the cytometer in standby mode.

Setting Up the Experiment

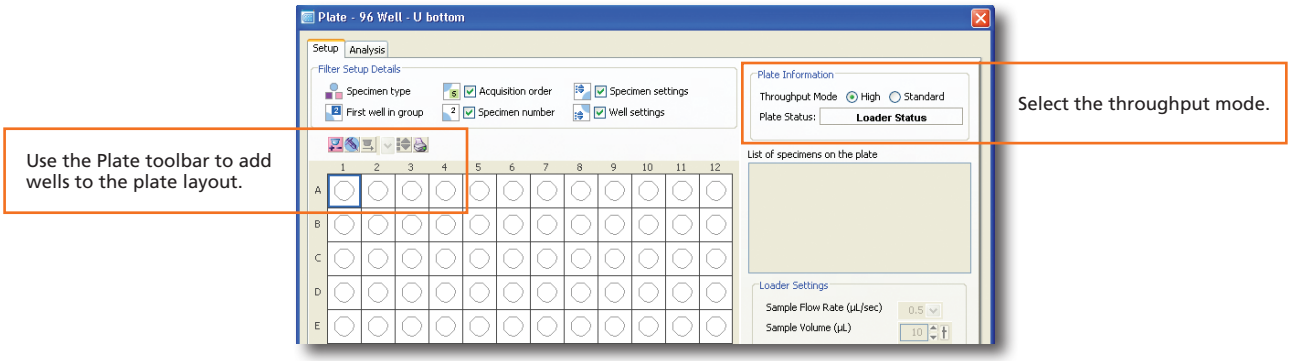
1. Create Browser elements.



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

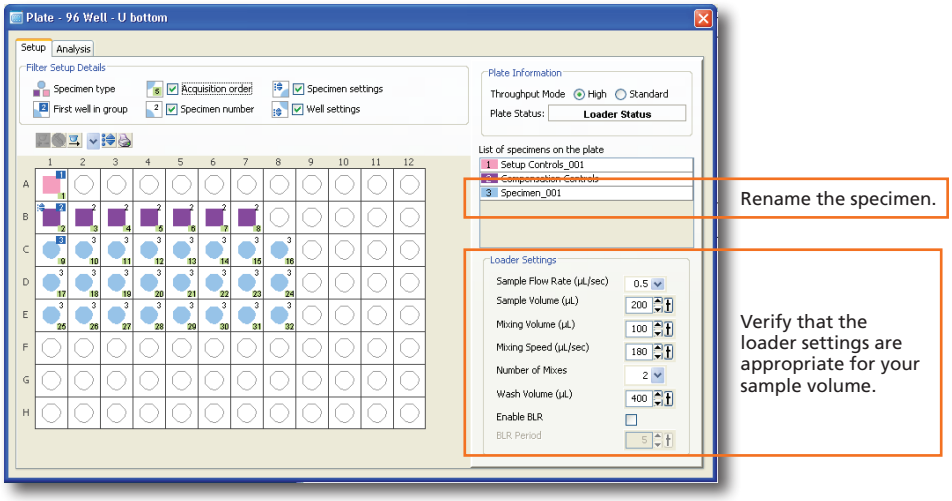


3. Create setup control wells.

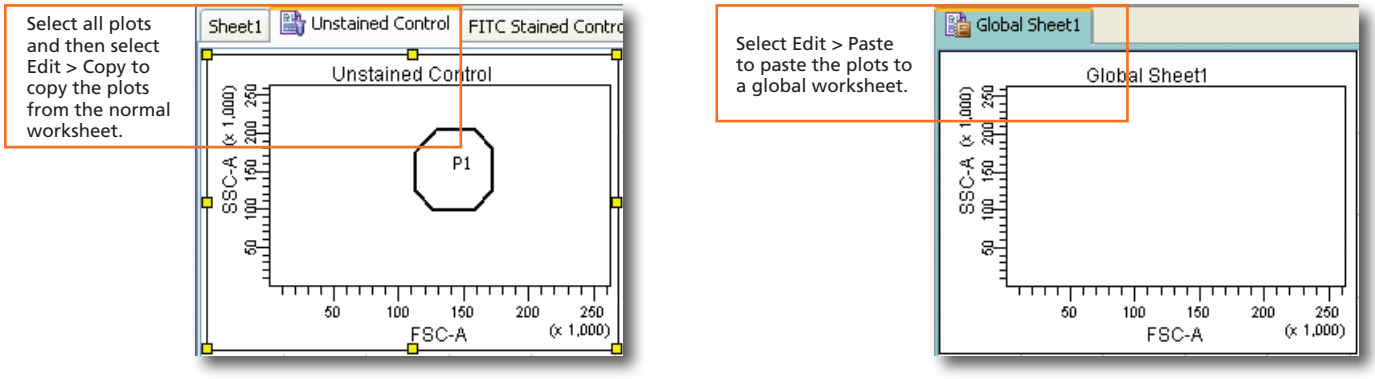


4. Select the first well for the compensation controls, right-click, and select Setup > Create Compensation Controls.

5. Create specimen wells.

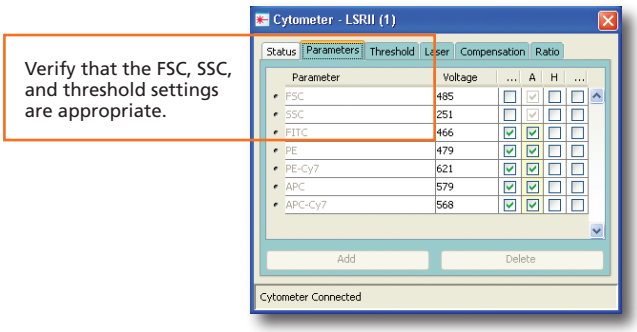


6. Create a global worksheet.



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

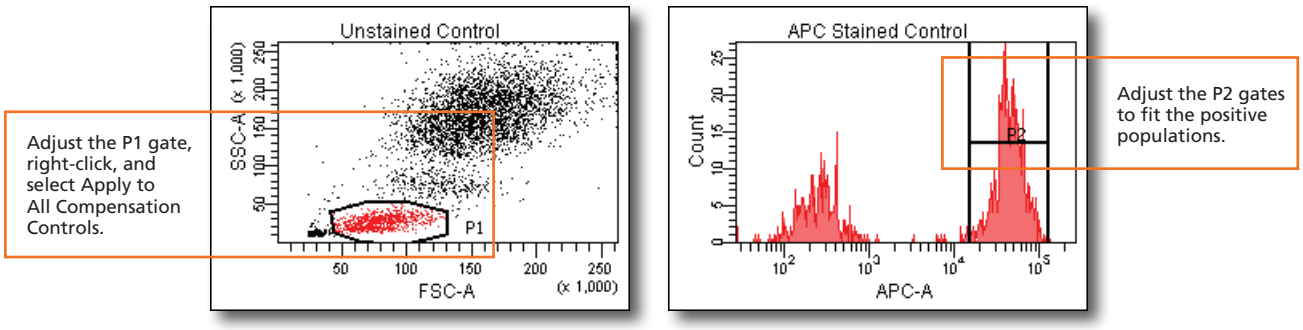
8. Select the Setup Control well and click Run Well(s) .



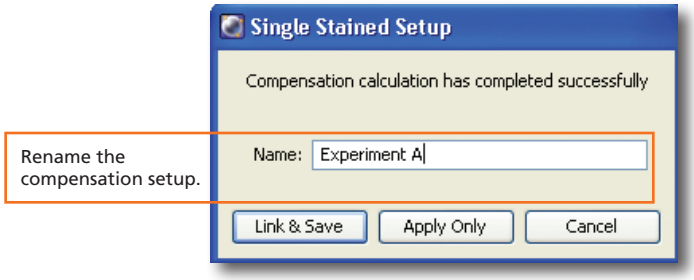
9. Select all the compensation control wells and click 

.

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

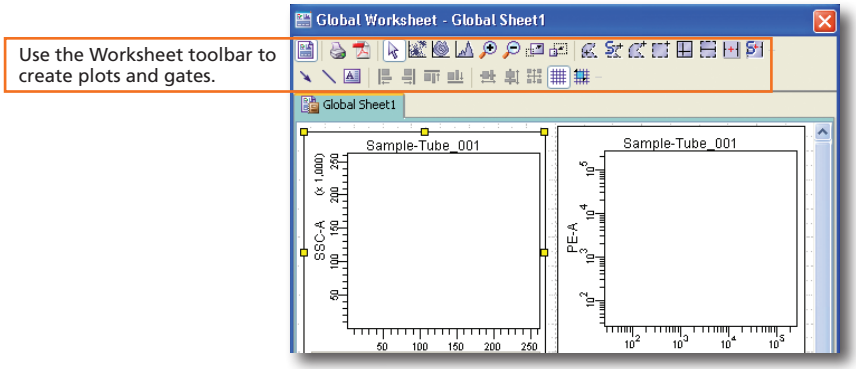


11. Select Experiment > Compensation Setup > Calculate Compensation.



Recording Specimen Data

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



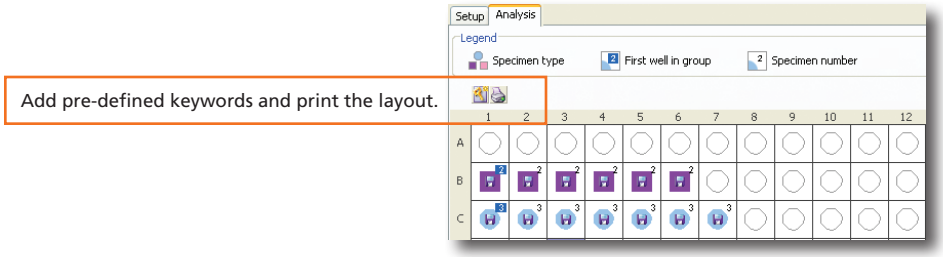
2. Select the first specimen well and click 

.

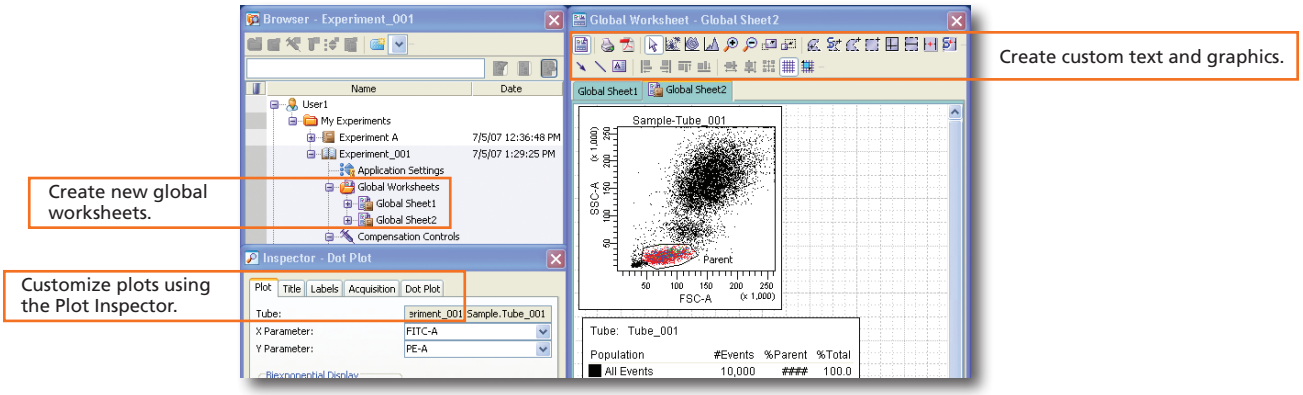
3. When recording is complete, place the cytometer in standby mode.

Analyzing Data

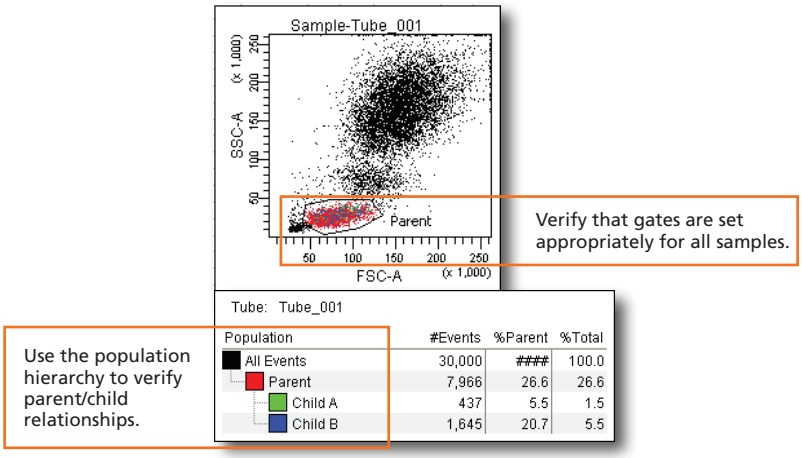
1. Under the Analysis tab of the Plate window, select a recorded well.



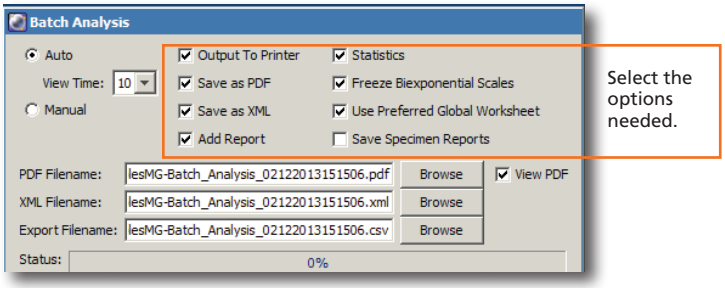
2. Create plots, gates, and statistics needed for analysis on a global worksheet.



3. Verify the analysis.

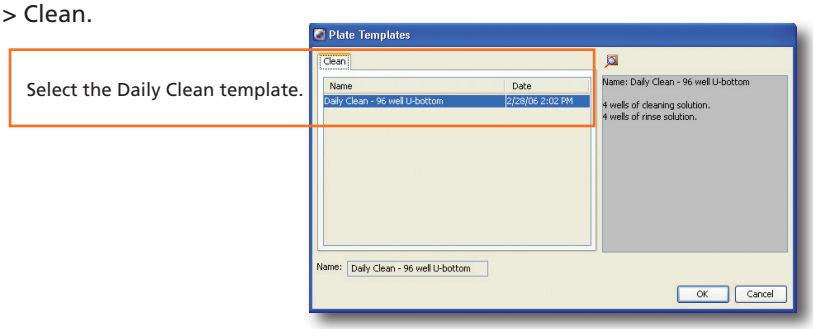


4. Right-click a specimen and select Batch Analysis.



Shutting Down the System

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

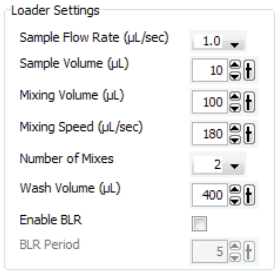


3. Install the prepared plate and click OK to begin cleaning.
4. Select File > Quit.
5. Turn off the cytometer and computer.

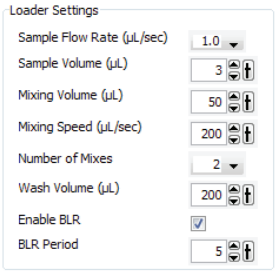
HTS Loader Settings Overview

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.

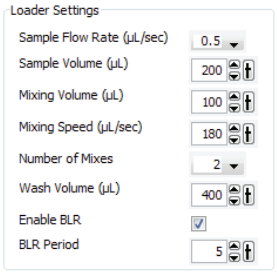
Default Loader Settings



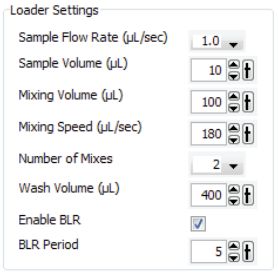
Specimen wells using Standard Throughput mode



Specimen wells using High Throughput mode



Setup Control wells



Compensation Control wells

Loader Setting	Description	Important Considerations
Sample Flow Rate	Amount of sample (in 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.	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 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.
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	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.