An overview of the Fortessa HTS and BD FACSDIVA software

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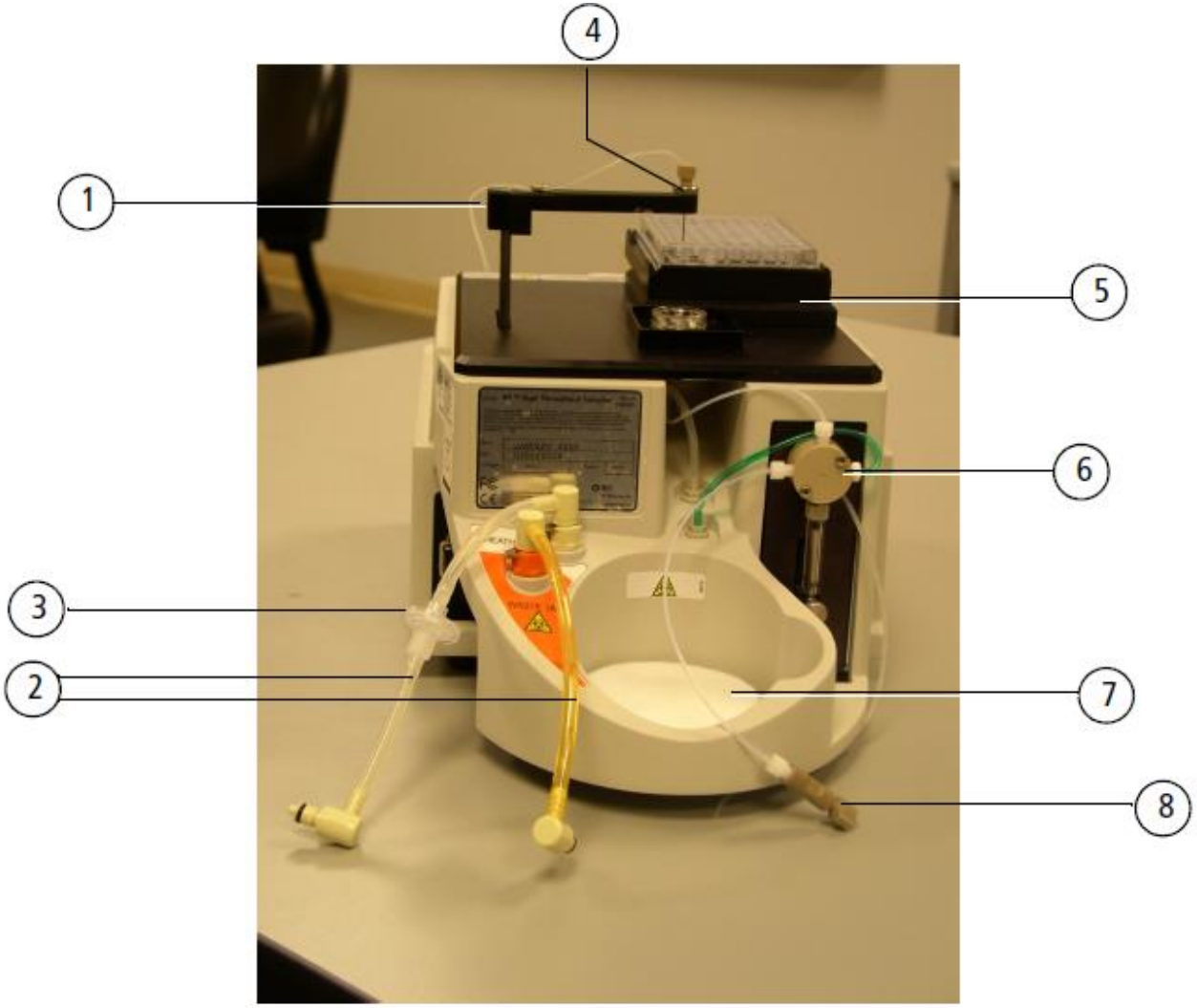
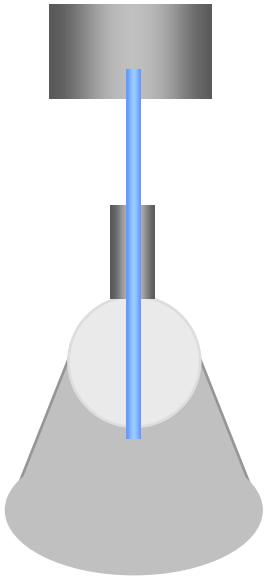
BD HTS



HTS Sample Introduction Sequence

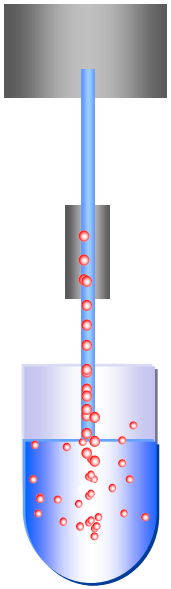
Flush Probe → **Mix and aspirate sample** → **Dispense sample into injection port** → **Deliver sample into flow cell**

HTS Sample Probe



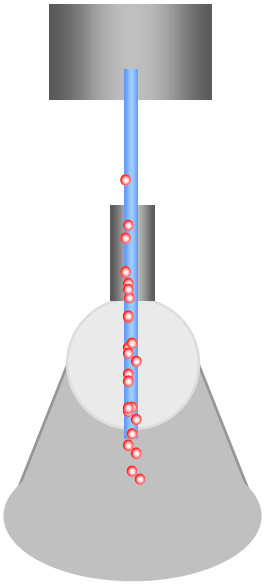
Wash Station/ Injection Port

HTS Sample Probe

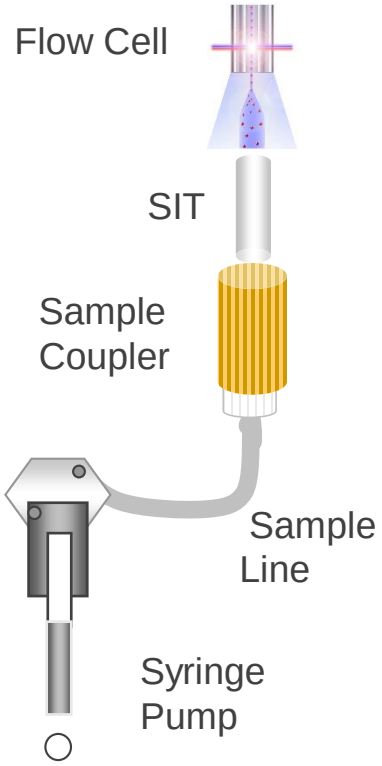


Sample Well

HTS Sample Probe



Wash Station/ Injection Port



Quick Reference Guide

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

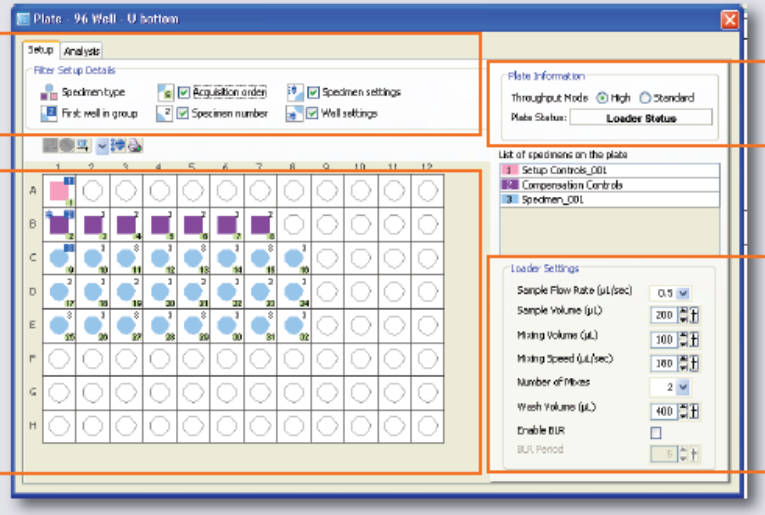


Plate Information
Designate throughput mode and view plate status.

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

Workflow Overview

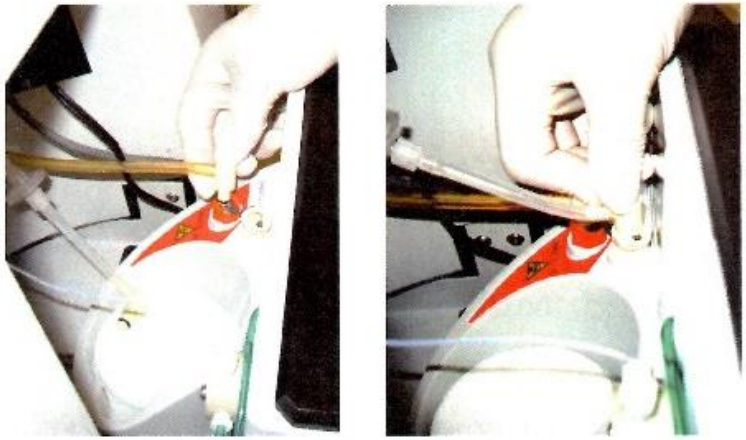
Before starting your daily workflow, ensure that your lab's software administrator has performed all the necessary tasks to set up the software for your use. This guide shows a workflow that uses application settings.

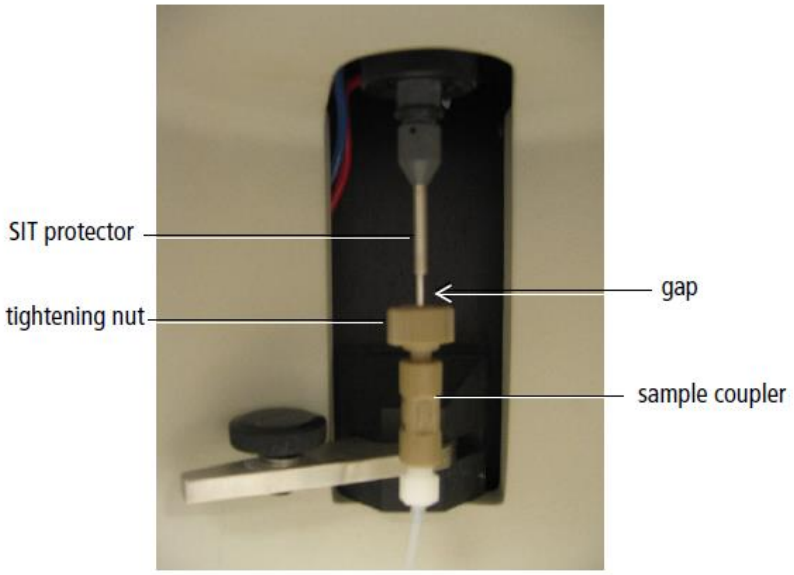
Starting up

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.

*1. Attach HTS.





Starting up

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.

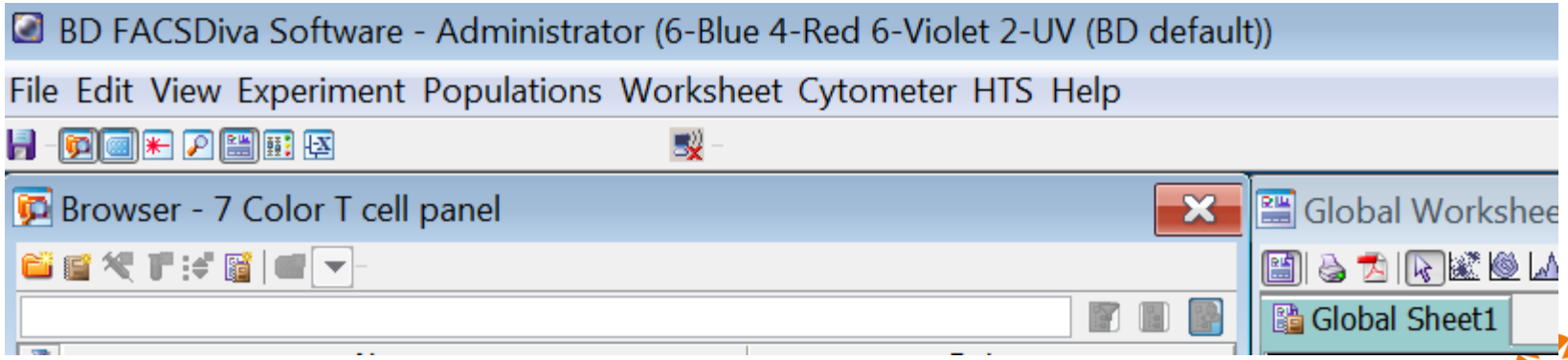
*1. Attach HTS.
2. Set acquisition mode switch to plate mode.

Starting up

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.

*1. Attach HTS.
2. Set acquisition mode switch to plate mode.
3. Prime the HTS 3 times using DIVA Software



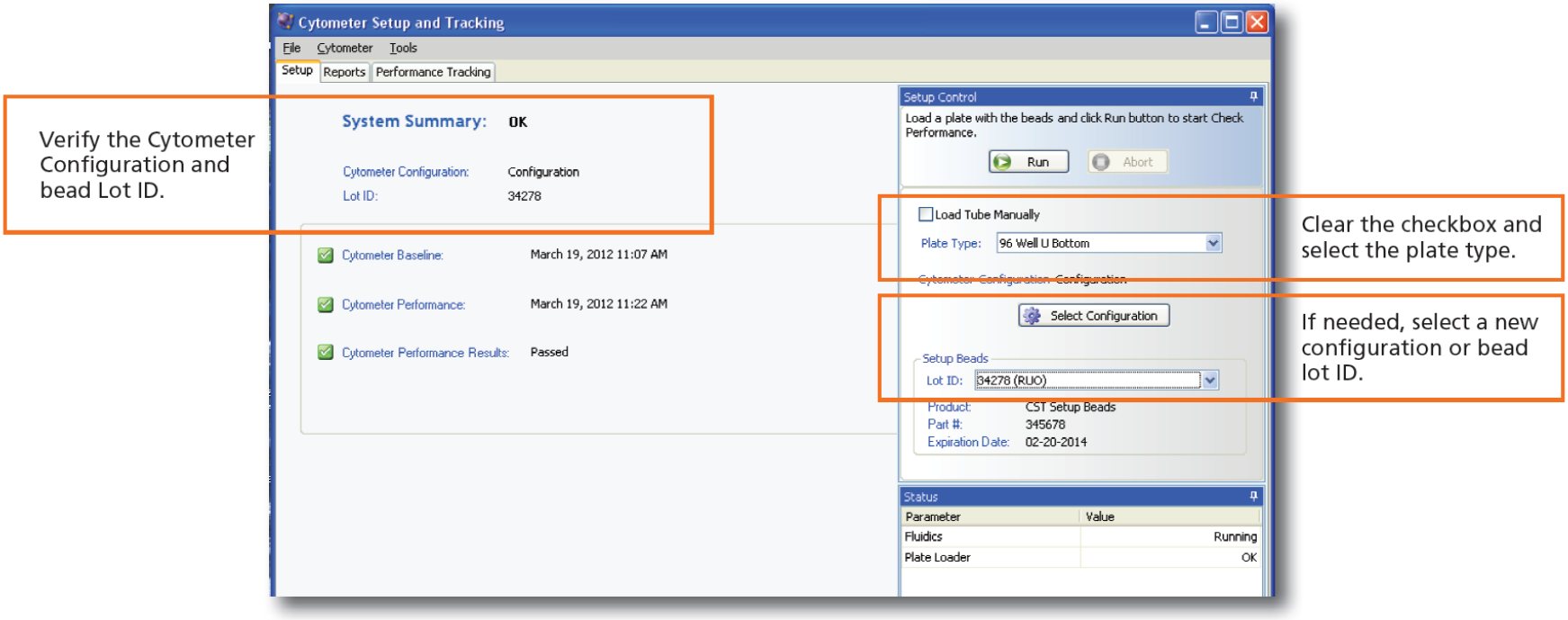
Performance Check (CS&T)

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.

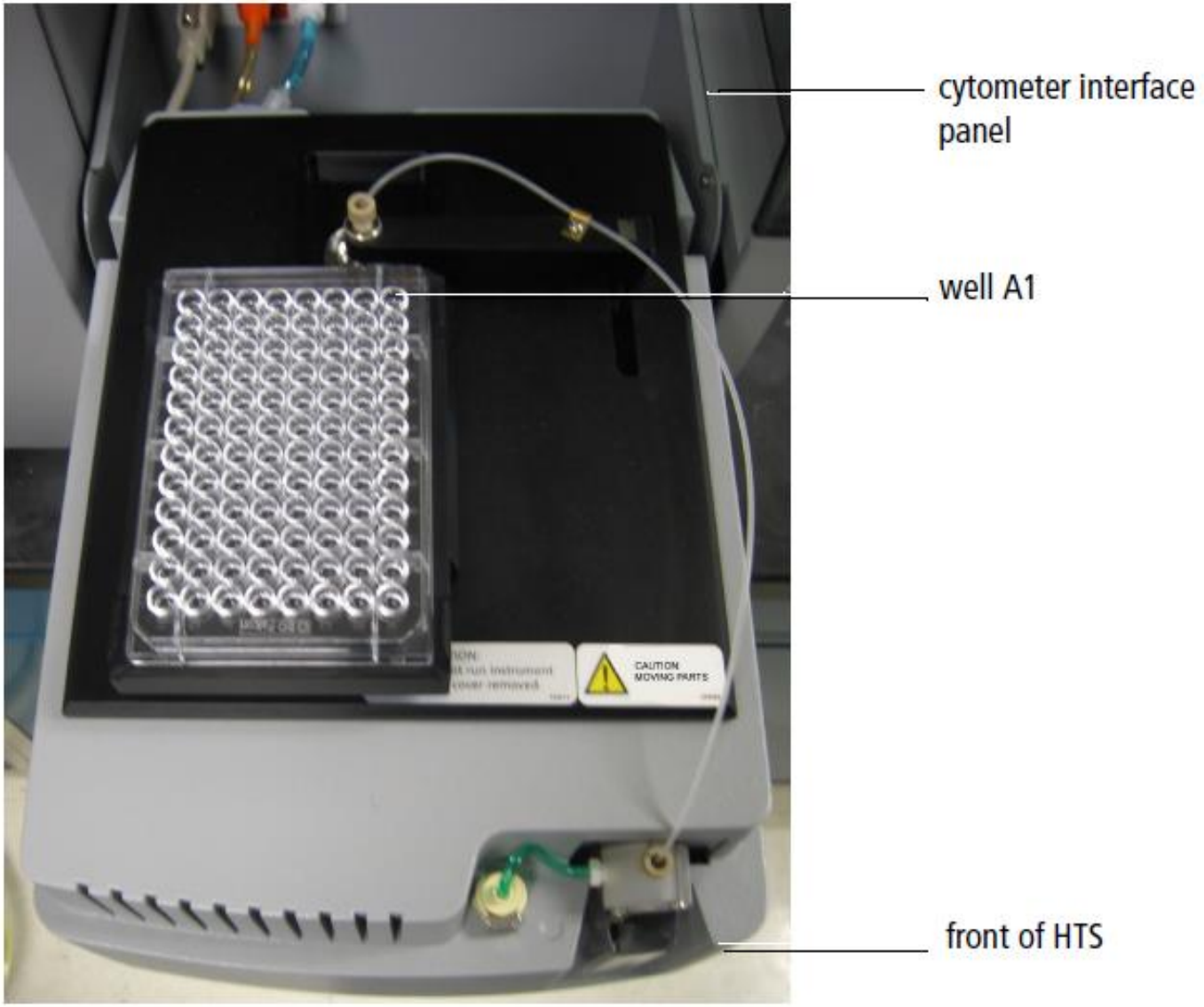
Checking Cytometer Performance

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.

*Load CS&T beads
(1 drop + 150 ml PBS)
in well A1



Cytometer Setup and Tracking (CS&T) System

- CS&T is a fully integrated system of software and reagents:
  - BD FACSDiva™ and FACSuite™ software
  - BD™ CS&T Beads
- Functions of the CS&T system:
  - Define and characterize instrument performance factors which can impact sensitivity and population resolution
    - The relative fluorescence detection efficiency (Qr)
    - The relative optical background (Br)
    - The electronic background noise in the system (SD_{EN})
  - Track cytometer performance
  - Standardize and automate cytometer setup
    - Application/Tube Settings

CS&T Benefits

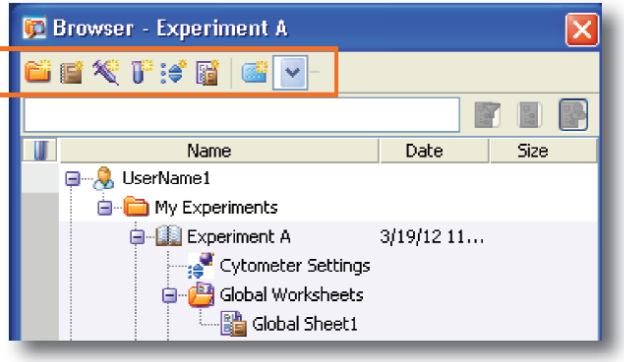
- Instrument Setup
  - Identifies any decrease in cytometer performance *early*
    - Helps identify the source of problems
  - Provides information, Electronic Noise (SD_{EN}), to help set up the instrument

- Multicolor Applications
  - Yields higher quality data from multicolor experiments
    - Provides consistent, reproducible data every day
      - Optimizes instrument setup for specific experimental conditions
    - Provides data on instrument performance at the time every experiment is run

Setting Up the Experiment

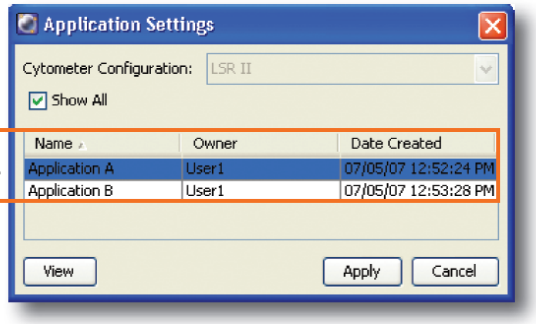
1. Create Browser elements.

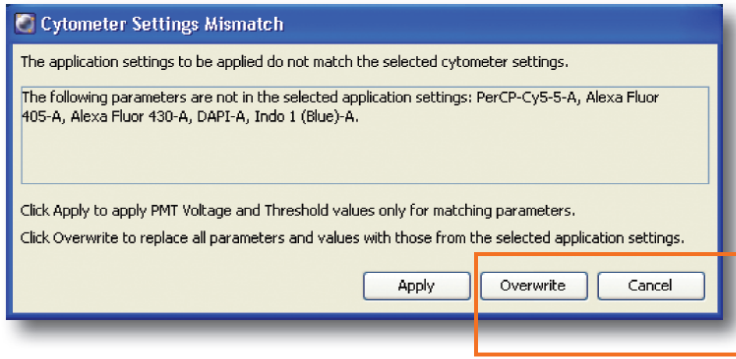
Use the Browser toolbar to add elements.



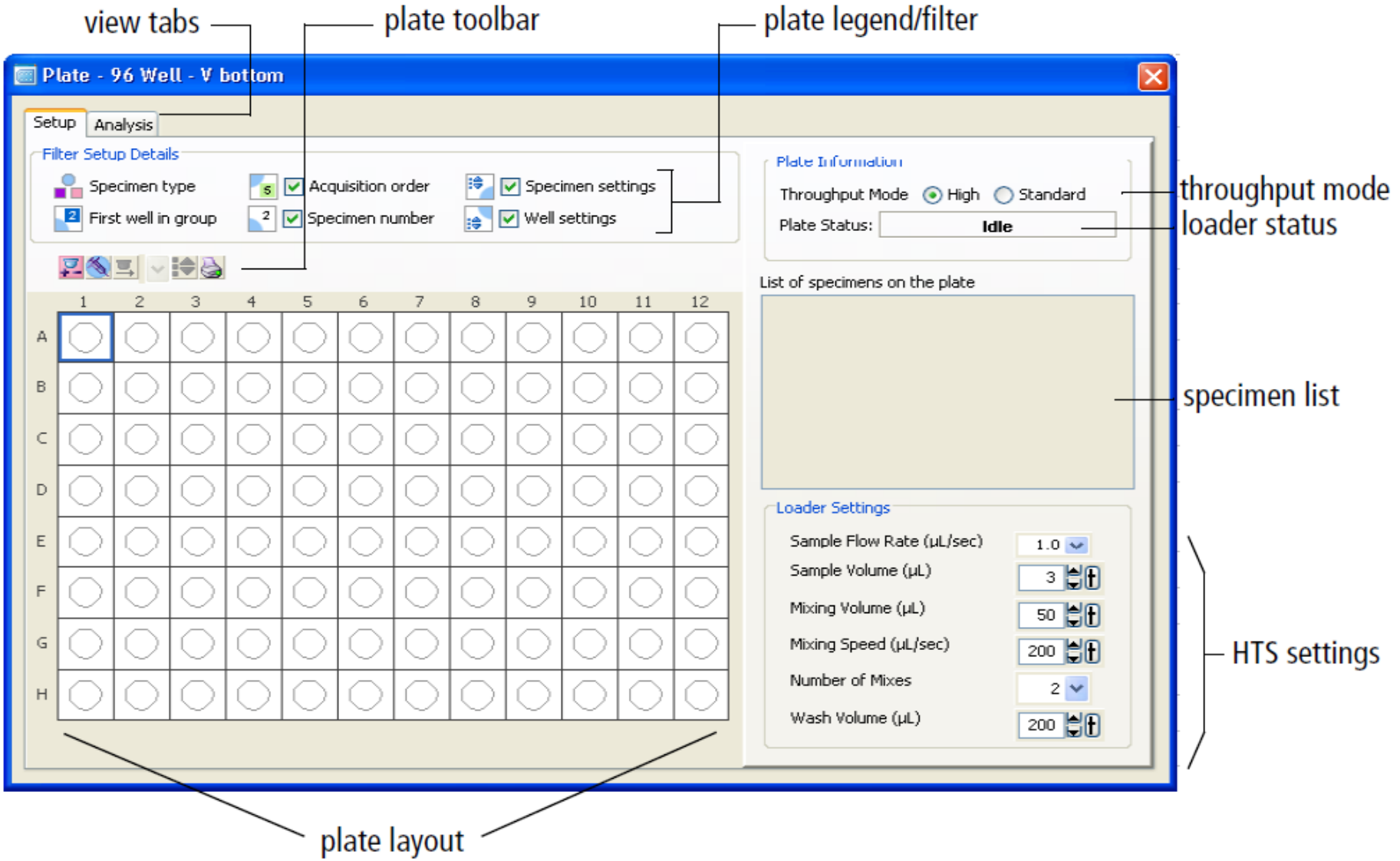
2. Right-click Cytometer Settings in the Browser. Select Application Settings > Apply.

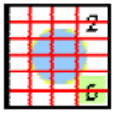
Select an application setting.



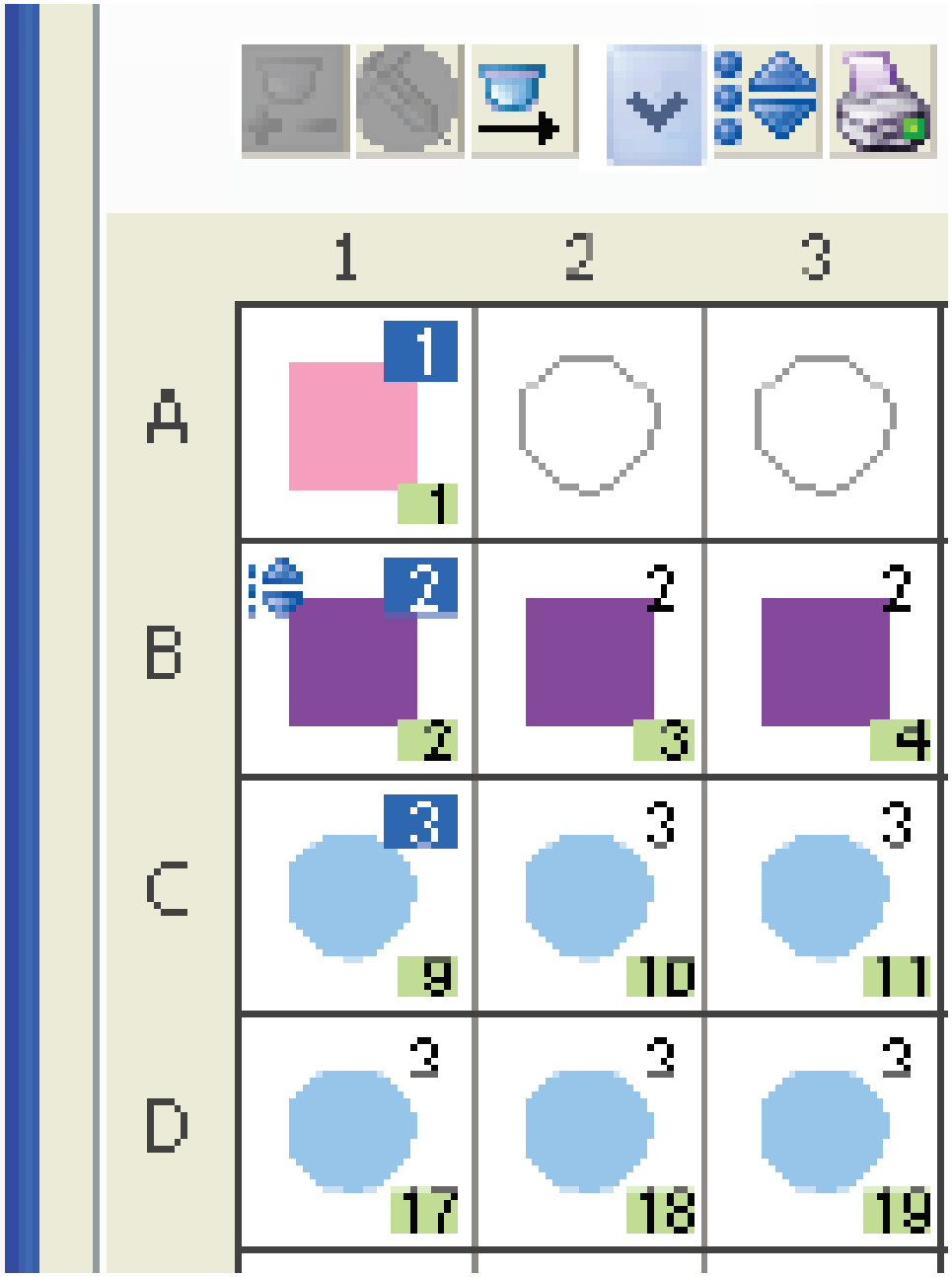


Click Overwrite if necessary.



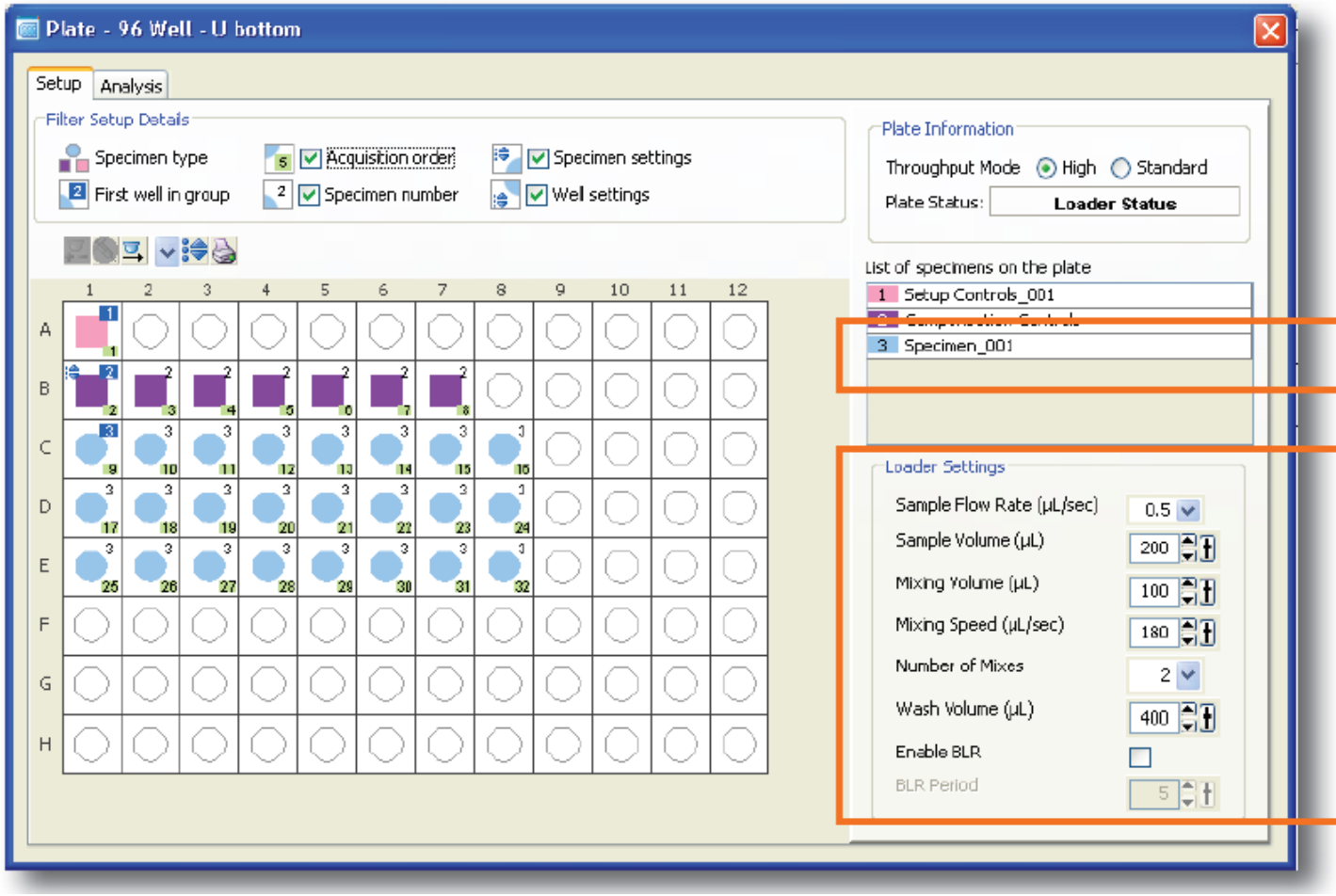
Well	Status
	well contains data—acquisition successful
	well contains no data
	well contains data—recording aborted
	well contains no data—acquisition aborted
	acquiring

	Specimen type – Indicates the type of control or sample assigned to a given well. The pink square represents a setup control, the purple square a compensation control, and the blue circle a specimen.
	First well in group – A dark blue square appears for the specimen number in the upper-right corner of the first well for each specimen.
	Acquisition order filter – The order (sequence number) in which each well will be acquired appears with a green background in the bottom-right corner of the well.
	Specimen number – The specimen number appears in the upper-right corner. Each well belonging to the same specimen will have the same specimen number.
	Specimen settings – When cytometer settings are added to a specimen, the cytometer settings icon appears in the upper-left corner of the well.
	Well settings – When cytometer settings are added to a well, the cytometer settings icon appears in the lower-left corner of the well.



Well types

- Setup wells (pink square)
  - Used to adjust PMT voltages
  - Fully stained samples, unstained samples
  - Compensation beads
- Compensation control wells (purple square)
  - Used to calculate and apply compensation
  - Single stained cells (or beads)
- Specimen wells (blue circles)
  - Used to collect data
  - Fully stained samples
  - e.g. biological replicates, treated vs untreated



Rename the specimen.

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

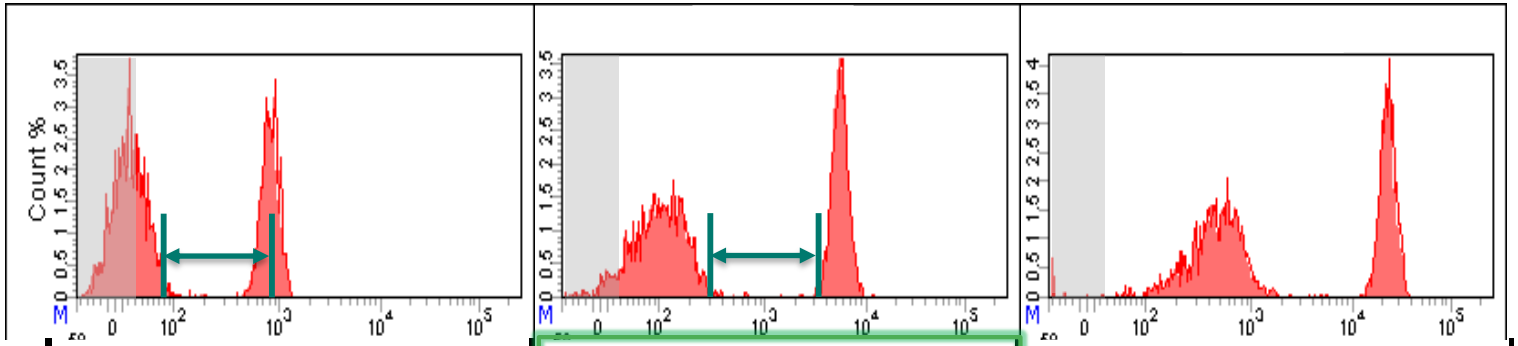
Set Up Experiment

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.

- Create an experiment
- Apply CS&T and application settings
- Define Parameters
- Optimize/adjust PMT voltages, if necessary
- Run and apply compensation

Adjusting PMTV to Maximize Resolution (SI)

FITC Detector (SD_{EN} = 20)

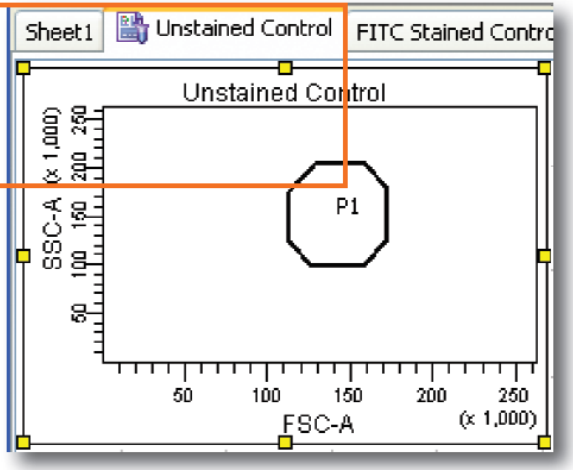


PMT Voltage	370	470	570
Stain Index	15	39	42
MFI Pos Cells	750	5072	21183
MFI Neg Cells	15	94	415
rSD Neg Cells	24	64	245

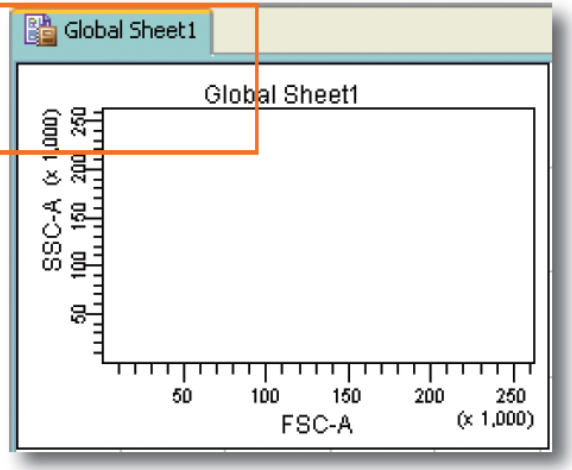
- Increasing the voltage from 370 to 470 significantly (2.6X) improves the resolution (Stain Index) in that detector.
- Increasing the voltage from 470 to 570 just increases the MFI of the positive and negative cells equivalently providing minimal improvement in resolution.
- ~470 volts is a good PMT setting. The rSD of the negative cells (64) is 2.5 times greater than the SDen.(20) [2.5 x 20 = 50]

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.

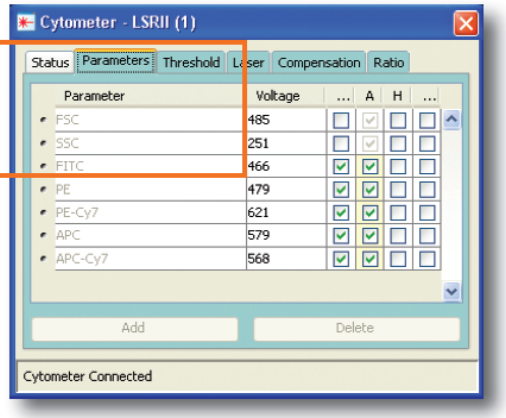


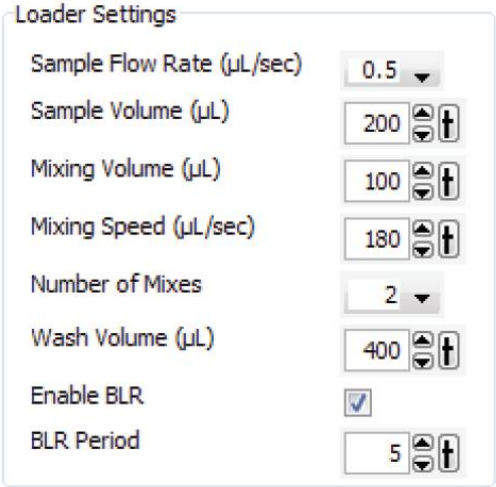
7. Install the prepared plate onto the HTS and place the cytometer in run mode.

8. Select the Setup Control well and click 

.

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





Setup Control wells

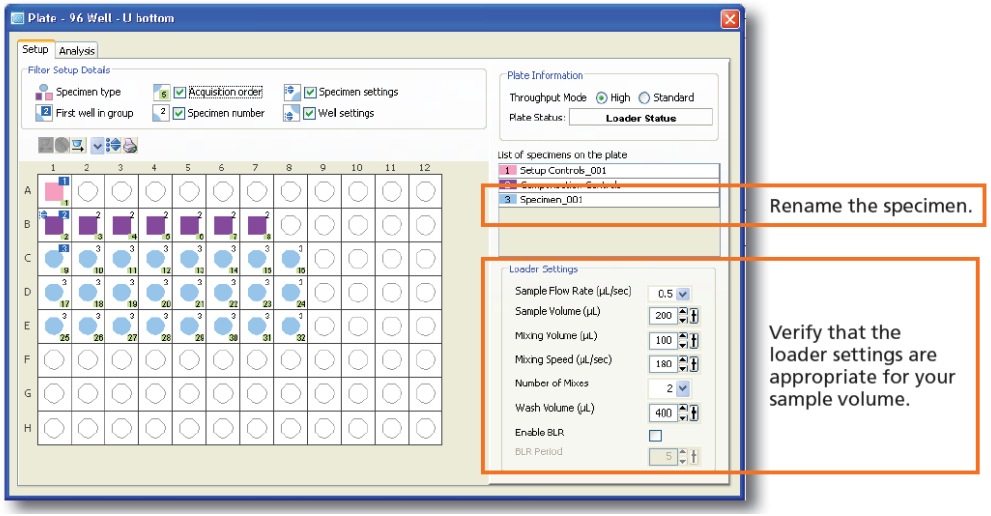
Set Up Experiment

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.

- Create an experiment
- Apply CS&T and application settings
- Define Parameters
- Optimize/adjust PMT voltages, if necessary
- Run and apply compensation

Create Compensation Controls

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



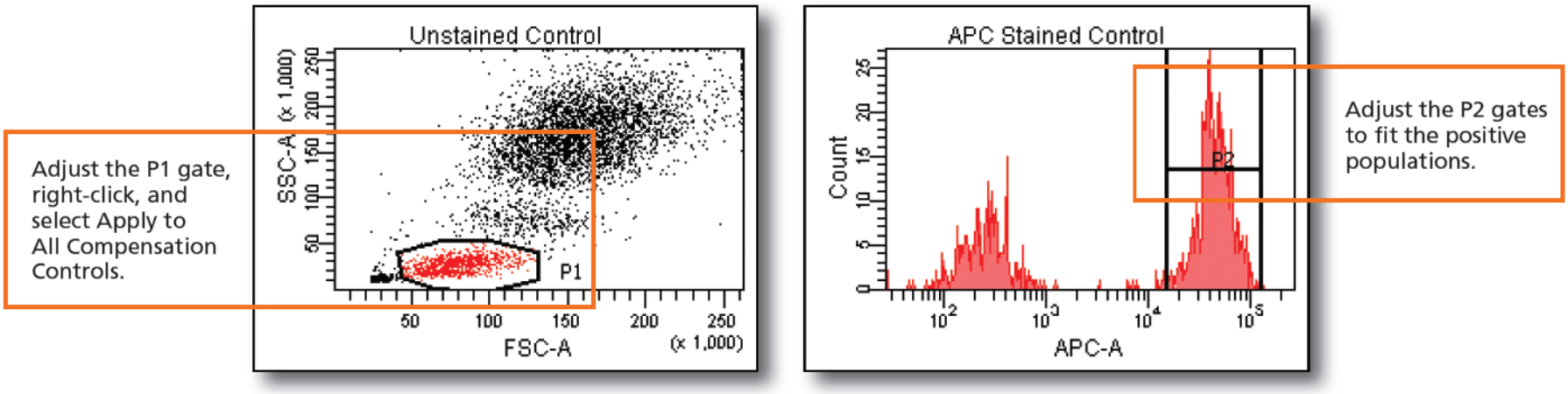
Notes:

- Adjust the PMT voltages using Setup Wells first.
- Compensation standards must appear in DIVA as they appear on the plate.
- First well is unstained control (or negative population), if using the universal negative.

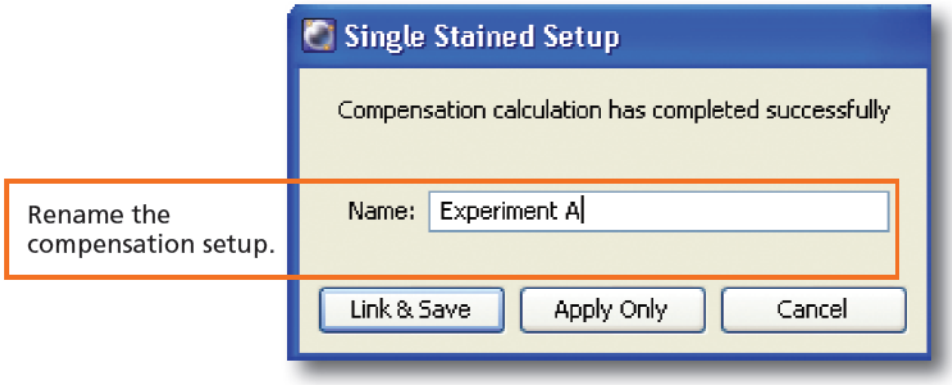
9. Select all the compensation control wells and click 

.

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



11. Select Experiment > Compensation Setup > Calculate Compensation.



Record data

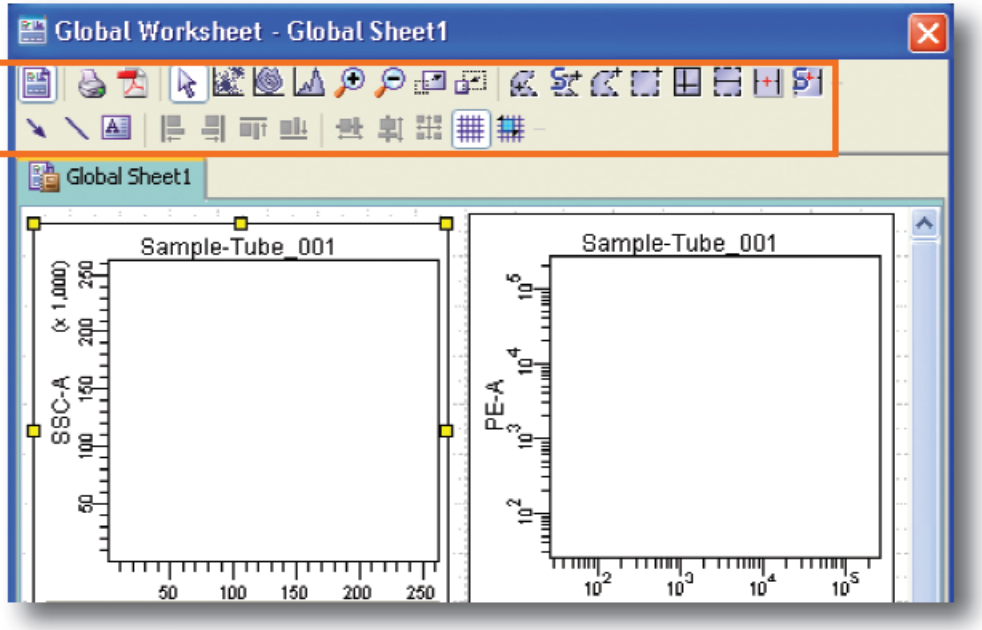
Start Up System ▶ Check Performance ▶ Set Up Experiment ▶ Record Data ▶ Analyze Data ▶ Shut Down System

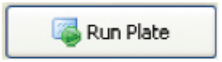
Before starting your daily workflow, ensure that your lab's software administrator has performed all the necessary tasks to set up the software for your use. This guide shows a workflow that uses application settings.

Recording Specimen Data

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

Use the Worksheet toolbar to create plots and gates.



2. Select the first specimen well and click 

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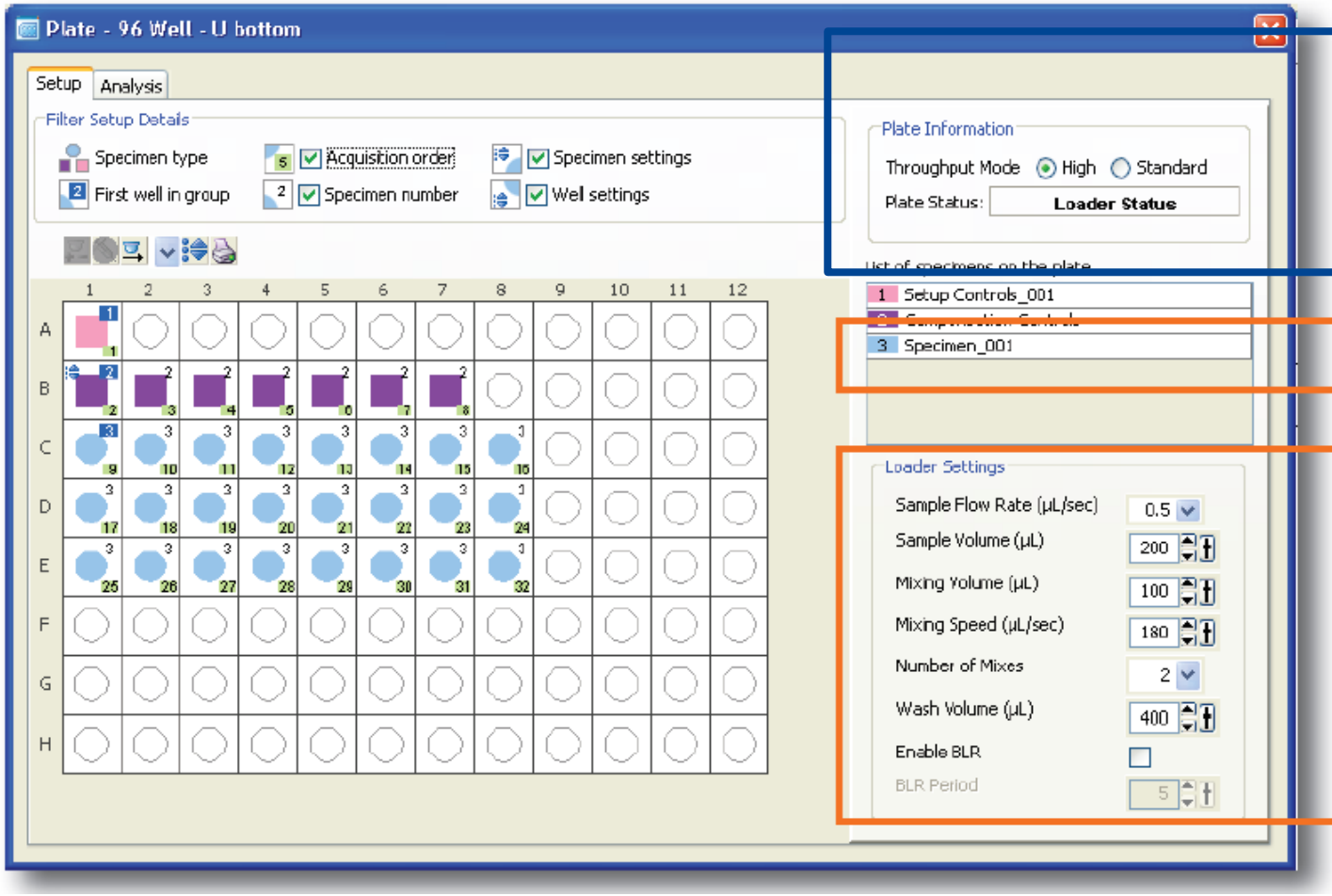
3. When recording is complete, place the cytometer in standby mode.



Rename the specimen.

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

Sampling Modes

	Standard Throughput	High Throughput
Approximate Processing Time for 96-well Plate with **Default Loader Settings**	44 min	15 min
Range for Sample Volume Acquired per Well	2–200 µL	2–10 µL

High-throughput mode uses the secondary pump to deliver sample to the flow cell, while the primary pump begins processing the next well.

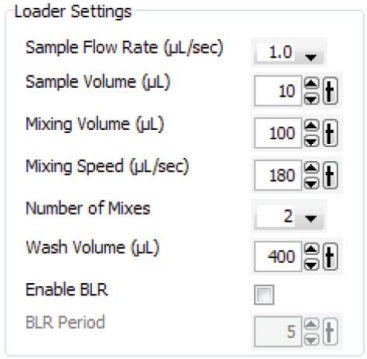
Setup and compensation control wells always use standard throughput—the throughput mode applies to all the other wells on the plate.

Loader Settings

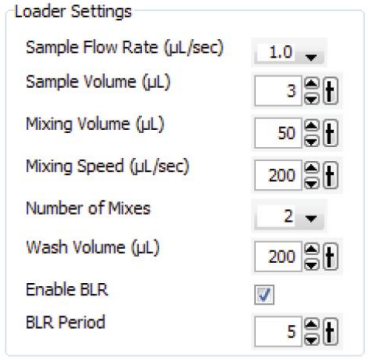
	Description
Sample Flow Rate (µL/sec)	Rate @ which sample is delivered to flow cell.
Sample Volume (µL)	Amount of sample delivered to the flow cell.
Mixing Volume (µL)	Amount of sample drawn up and down. To avoid bubbles, use no more than ½ of the total well volume.
Mixing Speed (µL/sec)	Mixing rate. Use a lower mixing rate for fragile samples.
Number of Mixes	Number of times the mixing volume is drawn up and down.
Wash Volume (µL)	Amount of sheath used to wash HTS between wells. Increase volume to reduce sample carry-over.
Enable BLR BLR Period	FACSDiva can be set to ignore the initial data for a period of time. The software multiplies the baseline restore (BLR) period by 10 to set the time in milliseconds. BLR = 5 equates to a delay of 50 milliseconds before recording data. Note that when you type in a value, you need to press the Enter key for the value to be saved.

Loader Settings

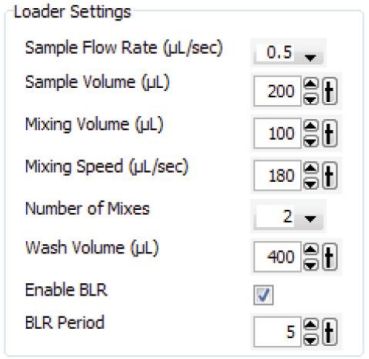
Default Loader Settings



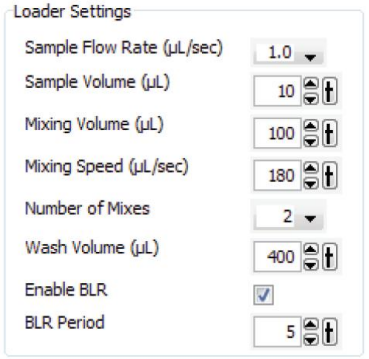
Specimen wells using Standard Throughput mode



Specimen wells using High Throughput mode



Setup Control wells



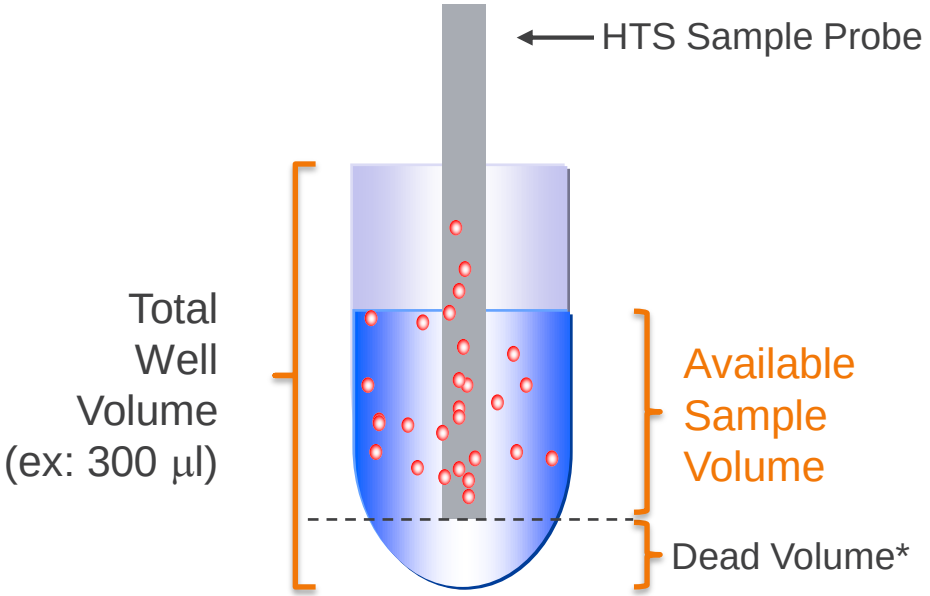
Compensation Control wells

Table 2-4 HTS settings for standard and high-throughput modes

Setting	Standard Mode		High-Throughput Mode	
	Default	Range	Default	Range
Sample flow rate (µL/sec)	1	0.5–3.0	1	0.5–3.0
Sample volume (µL)	10	2–200	2	2–10
Mixing volume (µL)[1]	100	5–100	50	5–100
Mixing speed (µL/sec)	180	25–250	200	25–250
Number of mixes (cycles)	2	0–5	2	0–5
Wash volume (µL)	400	200–800	200	200–800

Calculating Total Volume Needed

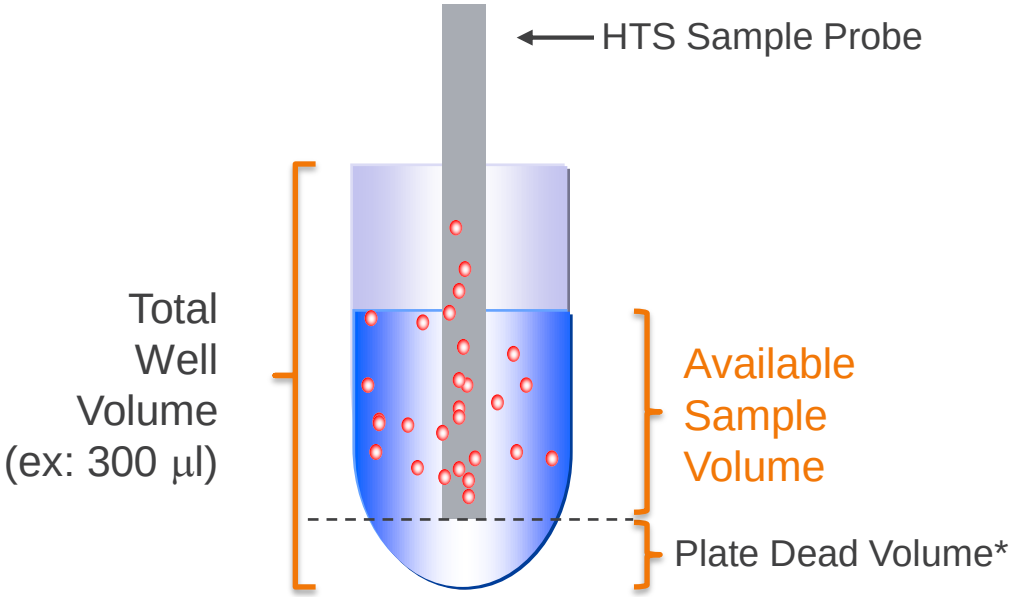
HTS dead volume = 20 µl
(Aspiration volume)



* Dead volume is plate-dependent.

Calculating Total Volume Needed

HTS dead volume = 20 µl
(Aspiration volume)



* Dead volume is plate-dependent.

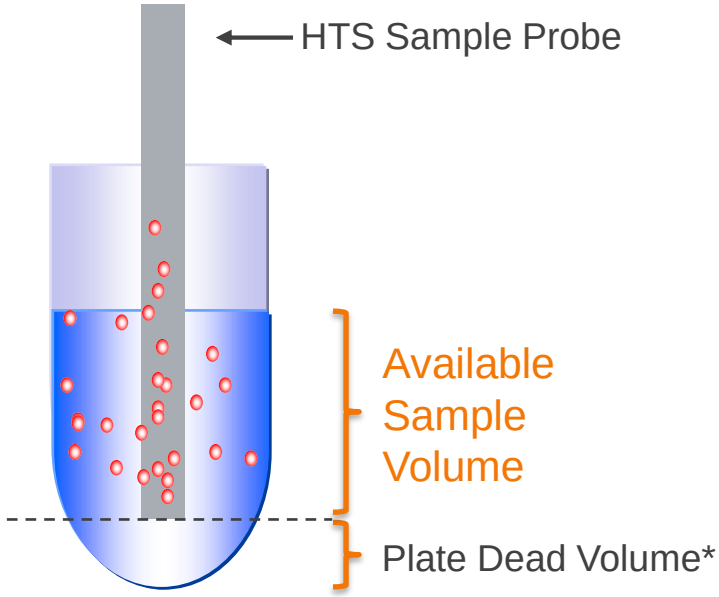
Total Volume Needed per well =

[*Sample Volume* + Aspiration Volume (20 µl)] x *Number of aspirations* + *plate dead volume*

[2µl sample + 20 µl overhead] x 1 + 30 µl dead volume = 52 µl for high throughput mode

Guidelines

	Minimum Total Well Volume (Guidelines)
High Throughput Mode	52 µl
Standard Mode	Sample volume + 50 µl



* Dead volume is plate-dependent.

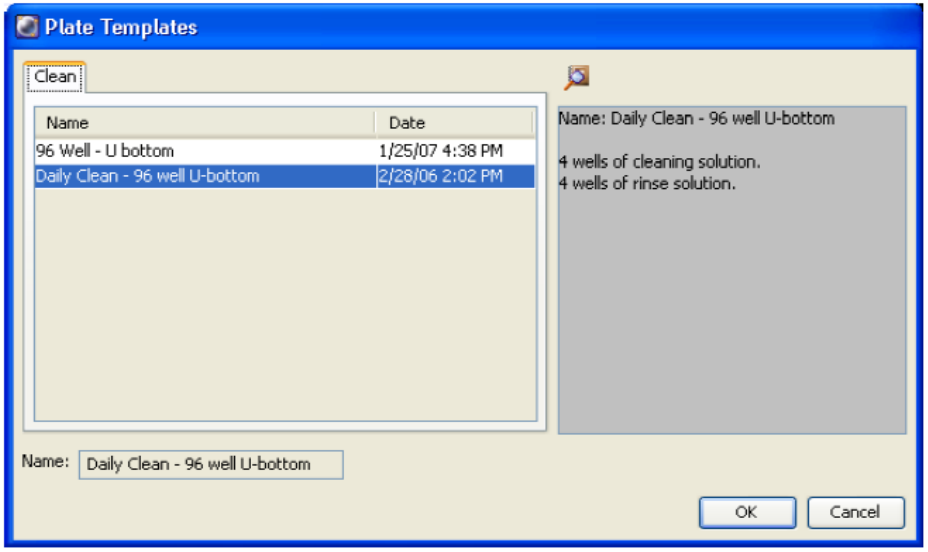
Shut down system

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.

1 Choose HTS > Clean.

The Plate Templates dialog appears (Figure 4-1 on page 105).

Figure 4-1 Plate Templates dialog



2 Select the *Daily Clean - 96 well U-bottom* template, if not already selected.

If you do not have a U-bottom plate for cleaning, you can set up your own cleaning template.

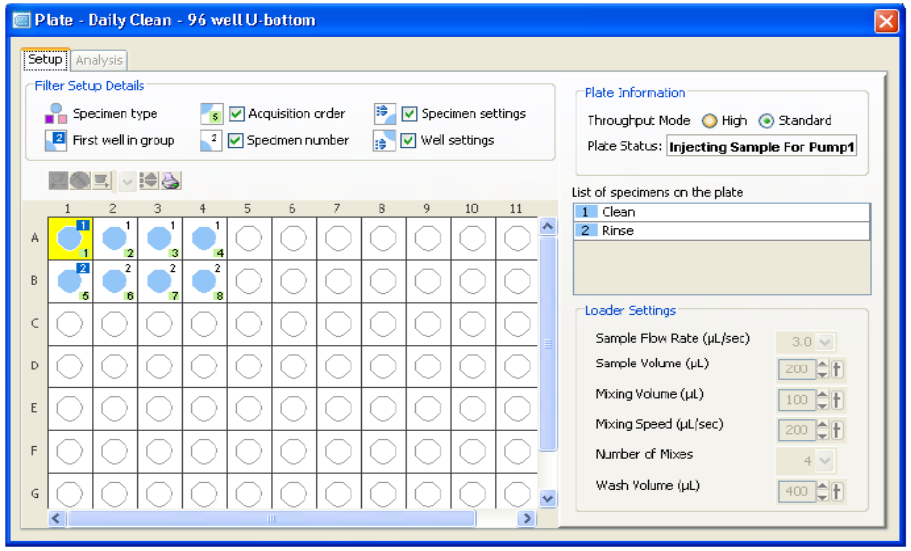
3 Click OK.

- The Plate Interface changes to show the Daily Clean Protocol view (Figure 4-2 on page 106).

Fill the wells of a 96-well plate according to the following table.

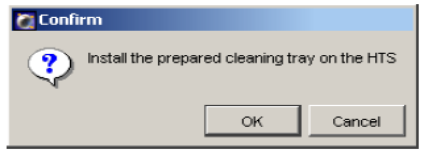
Wells	Solution	Volume (µL)
A1–A4	BD FACSClean[a]	200
B1–B4	DI water	200

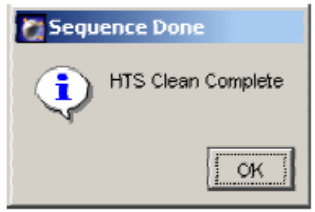
a. or a 10% bleach solution



- The following message appears.

Figure 4-3 Cleaning confirmation message





Put the end of the purging assembly line into a 500-mL beaker containing DI water.

Put the safety cover on the HTS.

Choose HTS > Prime; repeat nine times.

Priming will replace the sheath fluid with DI water.