



# An overview of the Fortessa HTS and BD FACSDIVA software

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APC-Cy7: US Patent 5,714,386

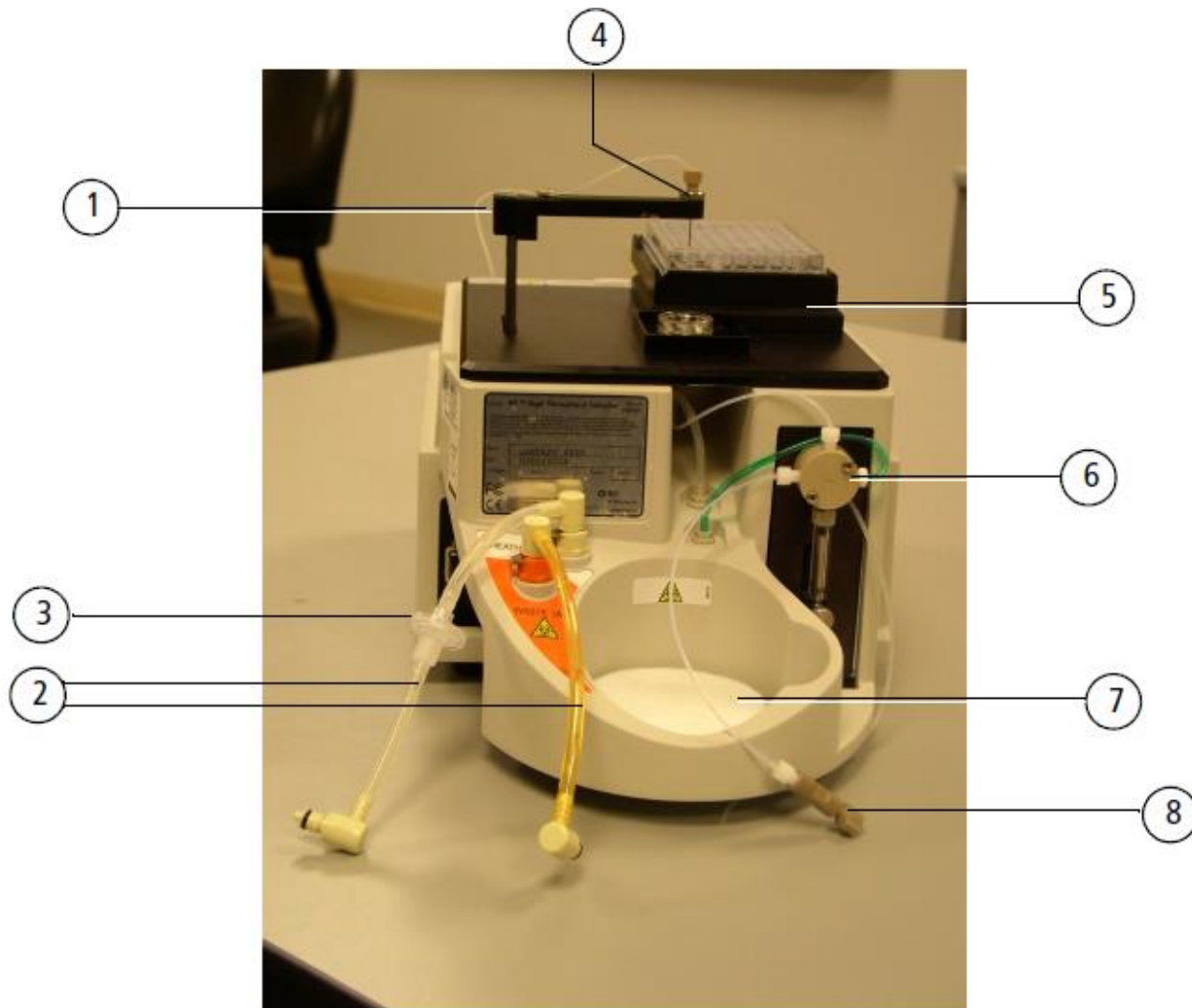
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# BD HTS



# HTS Sample Introduction Sequence

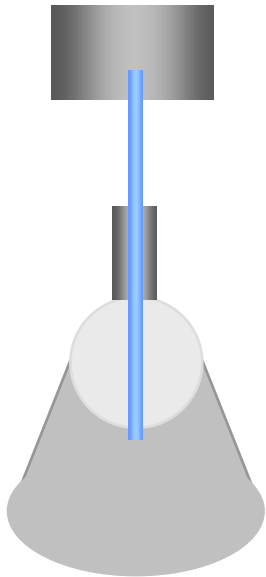
**Flush  
Probe**

**Mix and  
aspirate  
sample**

**Dispense  
sample into  
injection port**

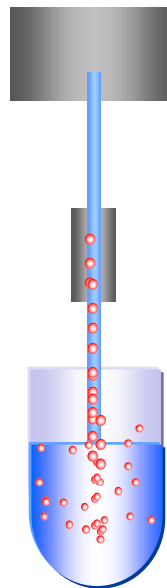
**Deliver  
sample into  
flow cell**

HTS Sample  
Probe



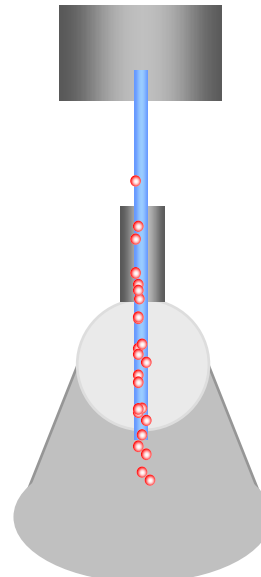
Wash Station/  
Injection Port

HTS Sample  
Probe

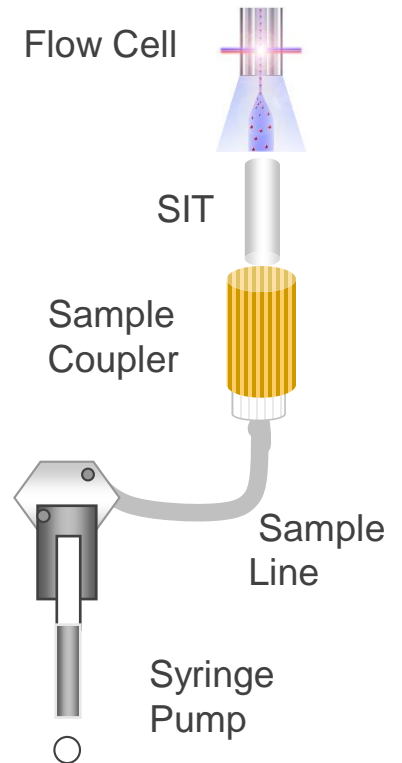


Sample Well

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.

The screenshot shows the 'Plate: 96 Well U bottom' window with the 'Setup' tab selected. The window is divided into several sections:

- Plate Setup Details:** Located at the top left, it includes checkboxes for 'Specimen type', 'Acquisition order', 'Specimen settings', 'First well in group', 'Specimen number', and 'Well settings'.
- Plate Information:** Located at the top right, it shows 'Throughput Mode' (High/Standard) and 'Plate Status' (Loader Status).
- Plate Layout:** The central 8x12 grid shows a 96-well plate layout with various wells highlighted in different colors (pink, purple, blue) to represent different specimen types or settings.
- Loader Settings:** Located at the bottom right, it includes fields for 'Sample Flow Rate (µL/sec)', 'Sample Volume (µL)', 'Mixing Volume (µL)', 'Mixing Speed (µL/sec)', 'Number of Mixes', 'Wash Volume (µL)', and 'Enable BUR'.

Four callout boxes provide additional context for these sections:

- 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, between-well washing, and acquisition delay.

# Workflow Overview



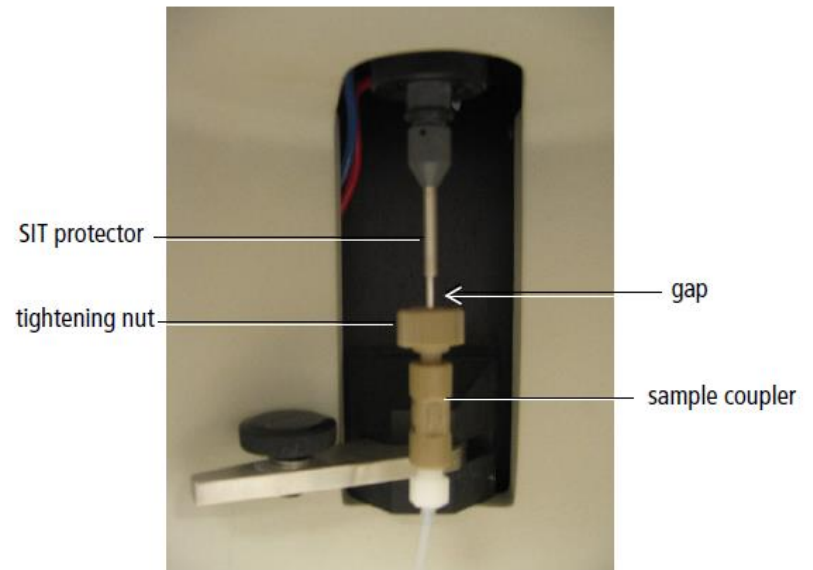
Before starting your daily workflow, ensure that your lab's software administrator has performed all the necessary tasks to set up the software for your use. This guide shows a workflow that uses application settings.

# 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. \*
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- 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

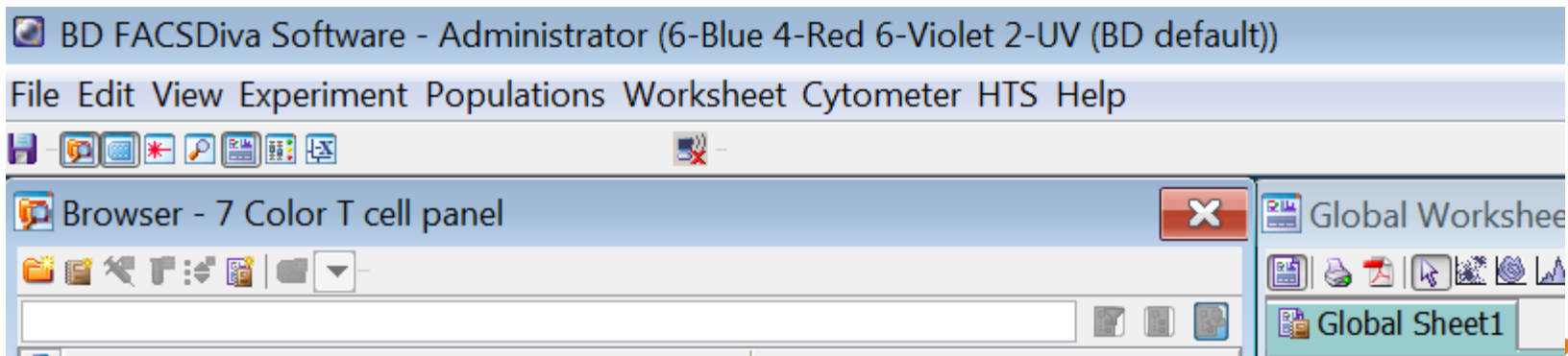
## Starting Up the System

- 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)



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.

The screenshot shows the 'Cytometer Setup and Tracking' software window. The 'System Summary' section is highlighted with an orange box and contains the text: 'Verify the Cytometer Configuration and bead Lot ID.' The 'Setup Control' section is also highlighted with an orange box and contains the text: 'Clear the checkbox and select the plate type.' The 'Setup Beads' section is highlighted with an orange box and contains the text: 'If needed, select a new configuration or bead lot ID.'

**System Summary: OK**

Cytometer Configuration: Configuration  
Lot ID: 34278

- ✓ Cytometer Baseline: March 19, 2012 11:07 AM
- ✓ Cytometer Performance: March 19, 2012 11:22 AM
- ✓ Cytometer Performance Results: Passed

**Setup Control**

Load a plate with the beads and click Run button to start Check Performance.

Load Tube Manually

Plate Type: 96 Well U Bottom

**Setup Beads**

Lot ID: 34278 (RUO)

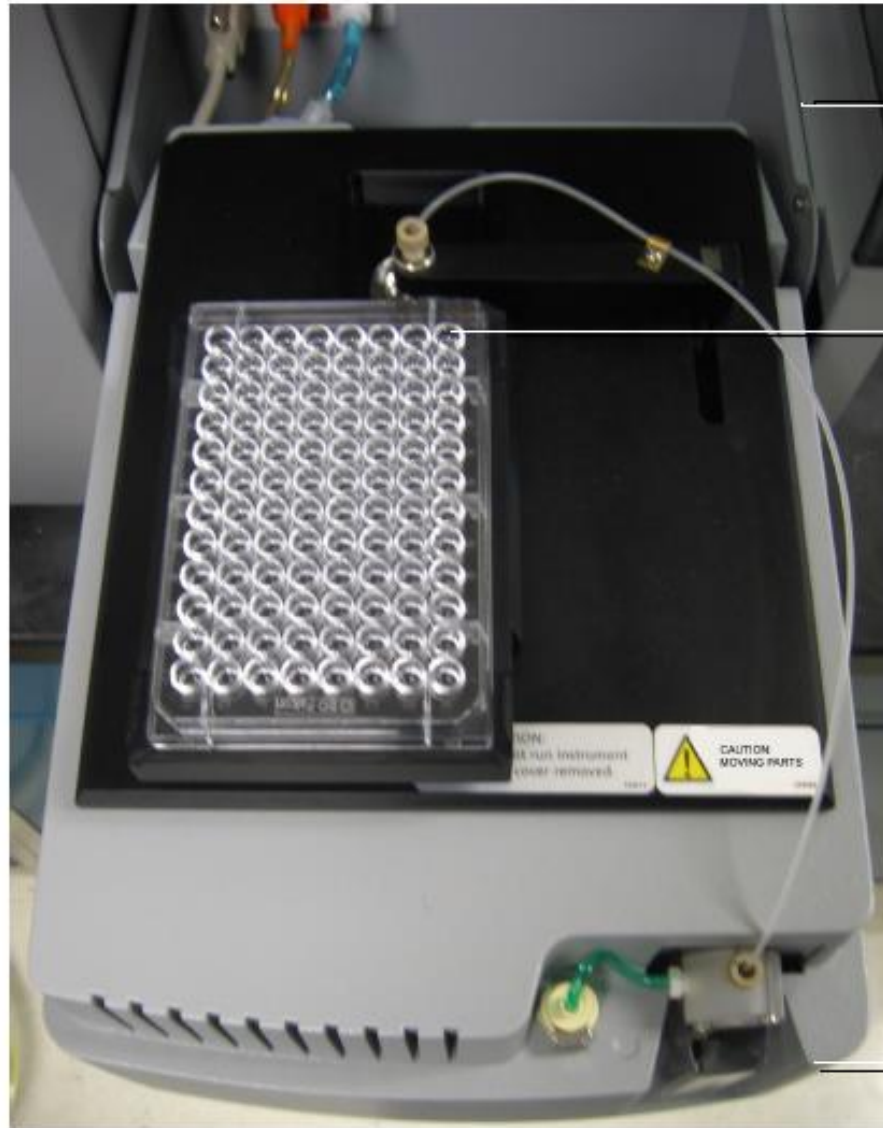
Product: CST Setup Beads  
Part #: 345678  
Expiration Date: 02-20-2014

**Status**

Parameter	Value
Fluidics	Running
Plate Loader	OK

- 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 interface panel

well A1

front of HTS

# 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 ( $Q_r$ )
    - 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

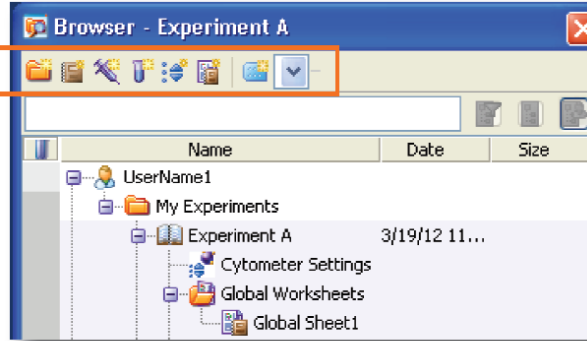
# CS&T Benefits

- 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

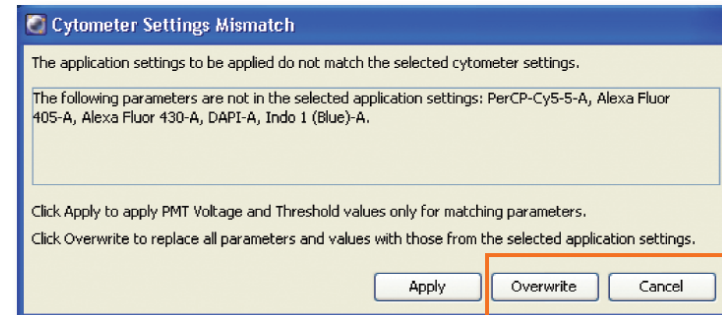
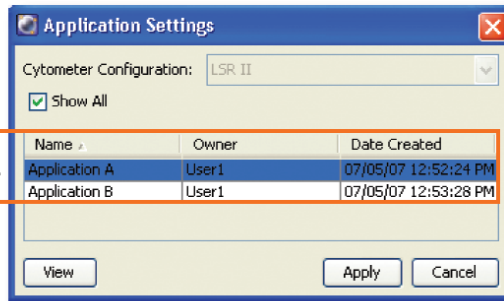
- 1 Create Browser elements.

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.

view tabs

plate toolbar

plate legend/filter

throughput mode

loader status

specimen list

HTS settings

plate layout

Plate - 96 Well - V bottom

Setup Analysis

Filter Setup Details

Specimen type

Acquisition order

Specimen number

Specimen settings

Well settings

Throughput Mode: High Standard

Plate Status: Idle

List of specimens on the plate

Sample Flow Rate (µL/sec): 1.0

Sample Volume (µL): 3


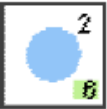

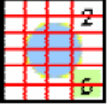

Mixing Volume (µL): 50

Mixing Speed (µL/sec): 200







Number of Mixes: 2

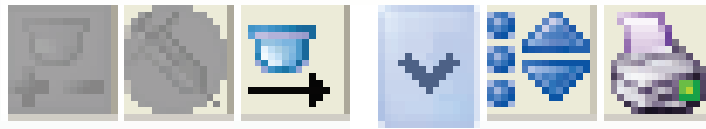
Wash Volume (µL): 200

	1	2	3	4	5	6	7	8	9	10	11	12
A	○	○	○	○	○	○	○	○	○	○	○	○
B	○	○	○	○	○	○	○	○	○	○	○	○
C	○	○	○	○	○	○	○	○	○	○	○	○
D	○	○	○	○	○	○	○	○	○	○	○	○
E	○	○	○	○	○	○	○	○	○	○	○	○
F	○	○	○	○	○	○	○	○	○	○	○	○
G	○	○	○	○	○	○	○	○	○	○	○	○
H	○	○	○	○	○	○	○	○	○	○	○	○

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



	<p>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.</p>
	<p>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.</p>
	<p>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.</p>
	<p>Specimen number – The specimen number appears in the upper-right corner. Each well belonging to the same specimen will have the same specimen number.</p>
	<p>Specimen settings – When cytometer settings are added to a specimen, the cytometer settings icon appears in the upper-left corner of the well.</p>
	<p>Well settings – When cytometer settings are added to a well, the cytometer settings icon appears in the lower-left corner of the well.</p>



	1	2	3
A			
B			
C			
D			

# 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

Plate - 96 Well - U bottom

Setup Analysis

Filter Setup Details

- Specimen type  Acquisition order  Specimen settings
- First well in group  Specimen number  Well settings

Plate Information

Throughput Mode  High  Standard

Plate Status: **Loader Status**

List of specimens on the plate

1	Setup Controls_001
2	Setup Controls_001
3	Specimen_001

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
- Enable BLR
- BLR Period 5

Rename the specimen.

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

# Set Up Experiment

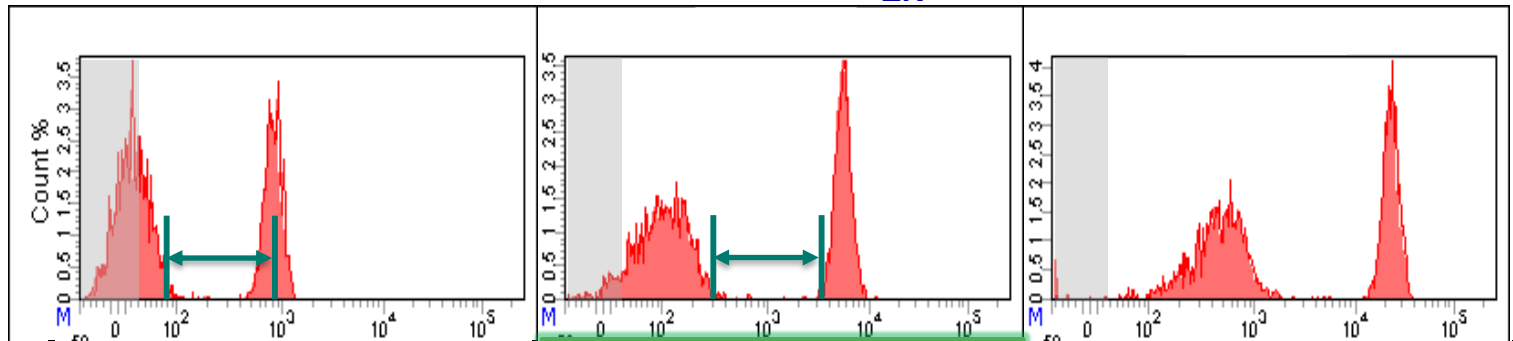


Before starting your daily workflow, ensure that your lab's software administrator has performed all the necessary tasks to set up the software for your use. This guide shows a workflow that uses application settings.

- 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)

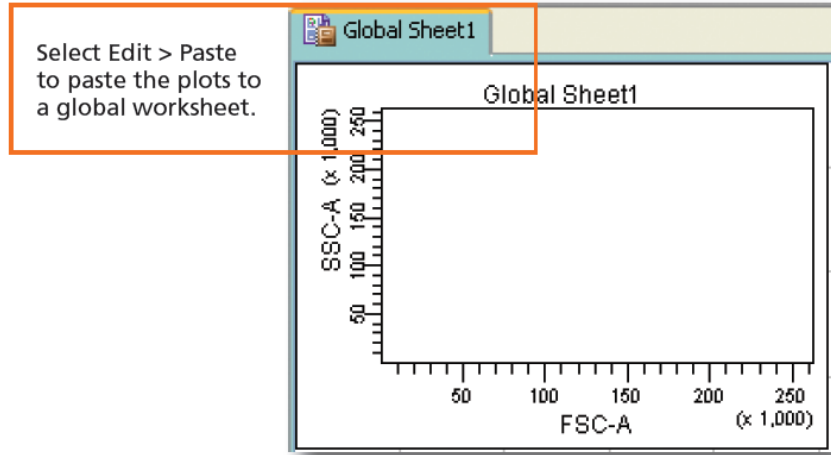
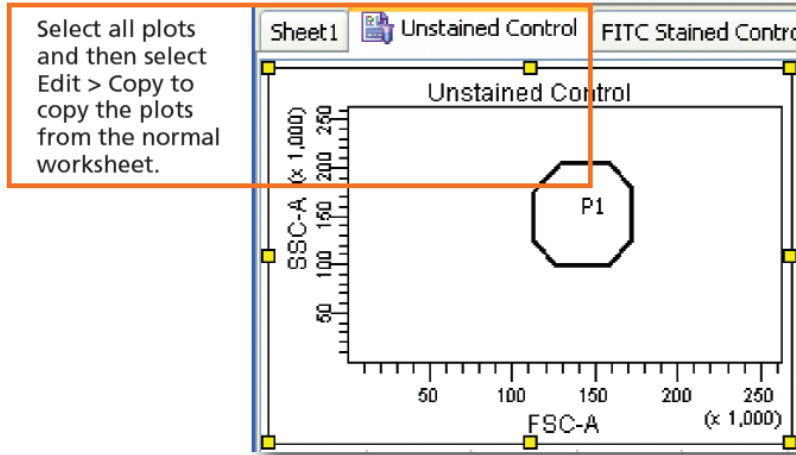
FITC Detector ( $SD_{EN} = 20$ )



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  $SD_{EN}$  (20) [ $2.5 \times 20 = 50$ ]

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 .

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

Cytometer - LSRII (1)

Status	Parameters	Threshold	User	Compensation	Ratio
	Parameter	Voltage	...	A	H
	FSC	485	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>
	SSC	251	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>
	FITC	466	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>
	PE	479	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>
	PE-Cy7	621	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>
	APC	579	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>
	APC-Cy7	568	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>

Add Delete

Cytometer Connected

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
- Enable BLR
- BLR Period 5

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.

The screenshot shows the 'Plate - 96 Well - U bottom' software interface. The 'Setup' tab is active, displaying a grid of 96 wells (A-H, 1-12). The 'Filter Setup Details' section includes options for 'Specimen type', 'Acquisition order', 'Specimen settings', 'First well in group', 'Specimen number', and 'Well settings'. The 'Plate Information' section shows 'Throughput Mode' set to 'High' and 'Plate Status' as 'Loader Status'. The 'List of specimens on the plate' section shows a list of specimens: '1 Setup Controls\_001', '2 Compensation Controls', and '3 Specimen\_001'. The 'Loader Settings' section includes fields for '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), 'Enable BLR' (unchecked), and 'BLR Period' (5). Two orange boxes highlight the specimen list and the loader settings.

Rename the specimen.

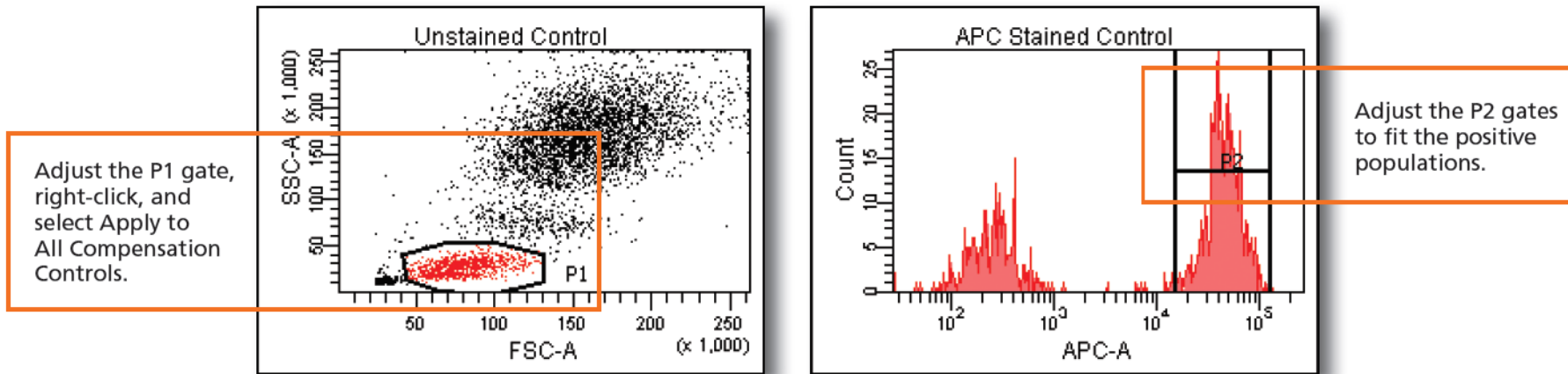
Verify that the loader settings are appropriate for your sample volume.

## Notes:

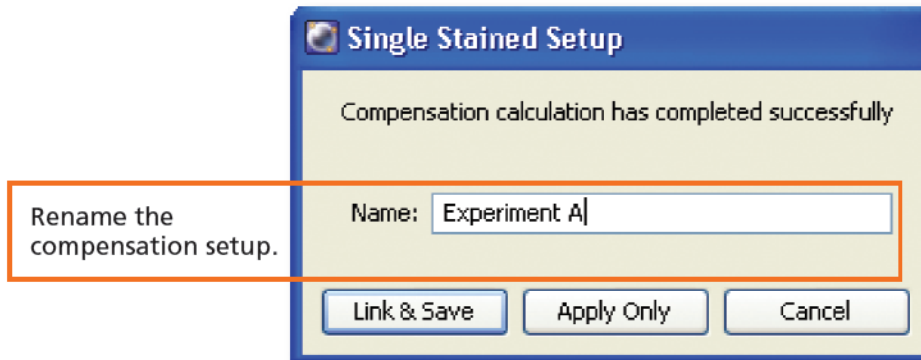
- Adjust the PMT voltages using Setup Wells first.
- Compensation standards must appear in DIVA as they appear on the plate.
- First well is unstained control (or negative population), if using the universal negative.

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.



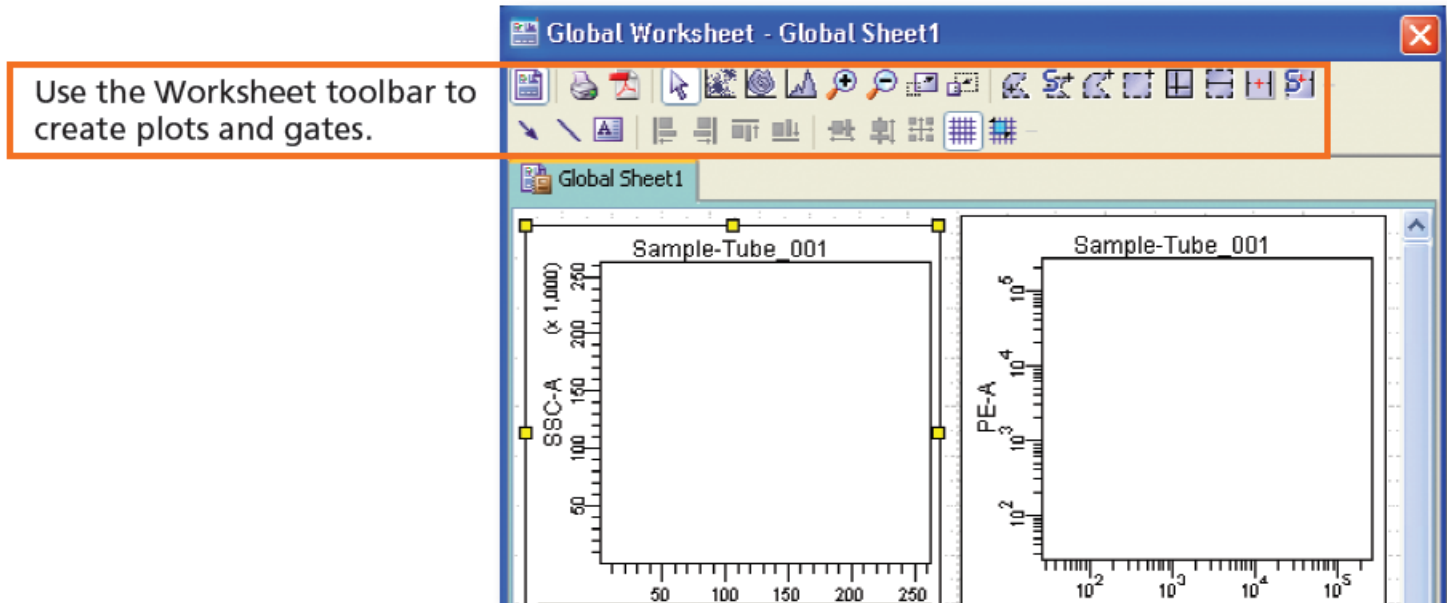
# Record data



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.



- 2 Select the first specimen well and click  .

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

Plate - 96 Well - U bottom

Setup Analysis

Filter Setup Details

Specimen type  Acquisition order  Specimen settings

First well in group  Specimen number  Well settings

	1	2	3	4	5	6	7	8	9	10	11	12
A	1											
B	2	2	2	2	2	2	2					
C	3	3	3	3	3	3	3	3	3			
D	3	3	3	3	3	3	3	3	3			
E	3	3	3	3	3	3	3	3	3			
F												
G												
H												

Plate Information

Throughput Mode  High  Standard

Plate Status: **Loader Status**

List of specimens on the plate

- 1 Setup Controls\_001
- 2 Compensation Controls
- 3 Specimen\_001

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

Enable BLR

BLR Period 5

Rename the specimen.

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

# Sampling Modes

	Standard Throughput	High Throughput
<b>Approximate Processing Time for 96-well Plate</b> with <u>Default Loader Settings</u>	44 min	15 min
<b>Range for Sample Volume Acquired per Well</b>	2–200 $\mu\text{L}$	2–10 $\mu\text{L}$

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 ( $\mu\text{L}/\text{sec}$ )

Rate @ which sample is delivered to flow cell.

Sample Volume ( $\mu\text{L}$ )

Amount of sample delivered to the flow cell.

Mixing Volume ( $\mu\text{L}$ )

Amount of sample drawn up and down. To avoid bubbles, use no more than  $\frac{1}{2}$  of the total well volume.

Mixing Speed ( $\mu\text{L}/\text{sec}$ )

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 ( $\mu\text{L}$ )

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

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
Enable BLR	<input type="checkbox"/>
BLR Period	5

Specimen wells using  
Standard Throughput mode

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
Enable BLR	<input checked="" type="checkbox"/>
BLR Period	5

Specimen wells using High  
Throughput mode

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
Enable BLR	<input checked="" type="checkbox"/>
BLR Period	5

Setup Control wells

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
Enable BLR	<input checked="" type="checkbox"/>
BLR Period	5

Compensation Control wells

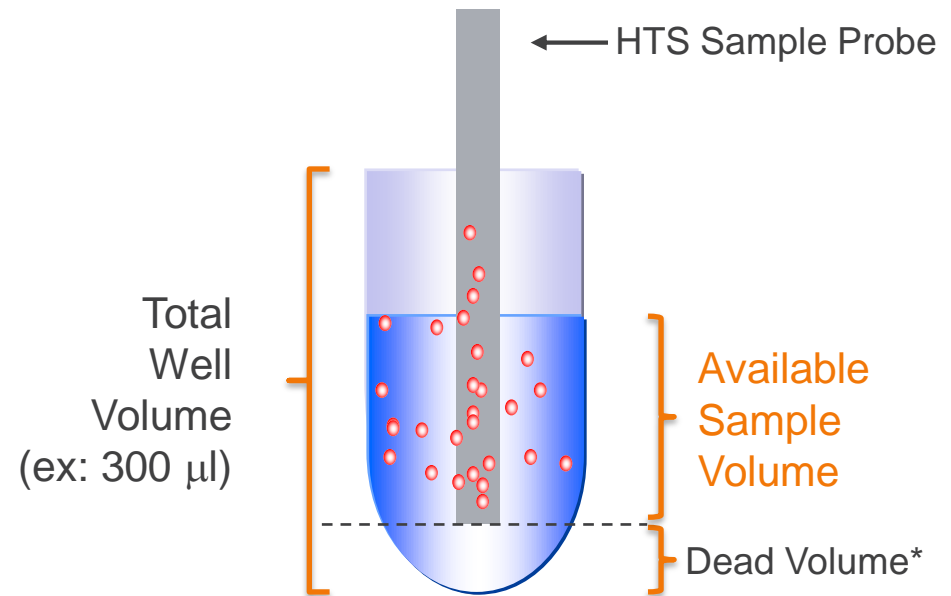


## Table 2-4 HTS settings for standard and high-throughput modes

Setting	Standard Mode		High-Throughput Mode	
	Default	Range	Default	Range
Sample flow rate ( $\mu\text{L}/\text{sec}$ )	1	0.5–3.0	1	0.5–3.0
Sample volume ( $\mu\text{L}$ )	10	2–200	2	2–10
Mixing volume ( $\mu\text{L}$ ) <sup>1</sup>	100	5–100	50	5–100
Mixing speed ( $\mu\text{L}/\text{sec}$ )	180	25–250	200	25–250
Number of mixes (cycles)	2	0–5	2	0–5
Wash volume ( $\mu\text{L}$ )	400	200–800	200	200–800

# Calculating Total Volume Needed

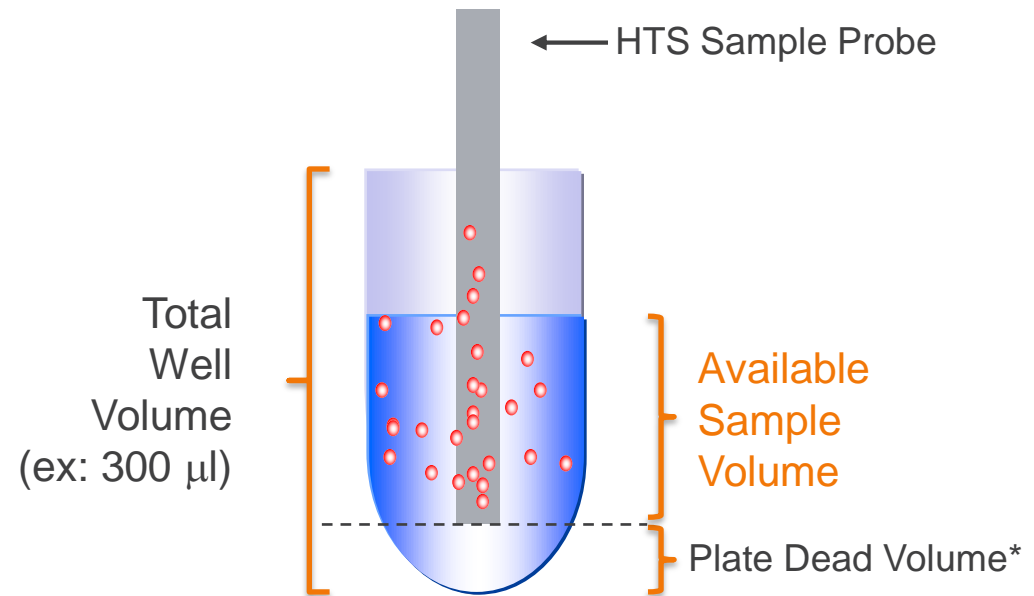
HTS dead volume = 20  $\mu\text{l}$   
(Aspiration volume)



\* Dead volume is plate-dependent.

# Calculating Total Volume Needed

HTS dead volume = 20  $\mu$ l  
(Aspiration volume)



\* Dead volume is plate-dependent.

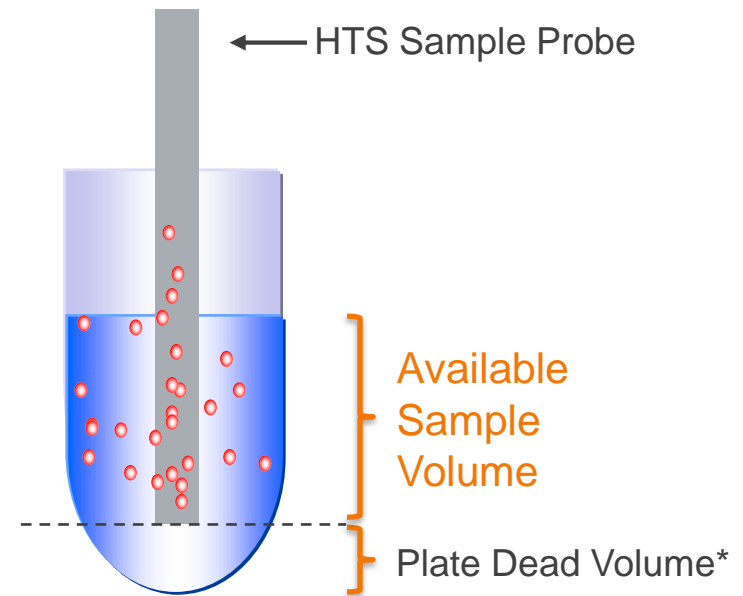
Total Volume Needed per well =

[*Sample Volume* + Aspiration Volume (20  $\mu$ l)] x *Number of aspirations* + *plate dead volume*

[2 $\mu$ l sample + 20  $\mu$ l overhead] x 1 + 30  $\mu$ l dead volume = 52  $\mu$ l for high throughput mode

# Guidelines

	Minimum Total Well Volume (Guidelines)
High Throughput Mode	52 $\mu$ l
Standard Mode	Sample volume + 50 $\mu$ l



\* Dead volume is plate-dependent.

# Shut down system

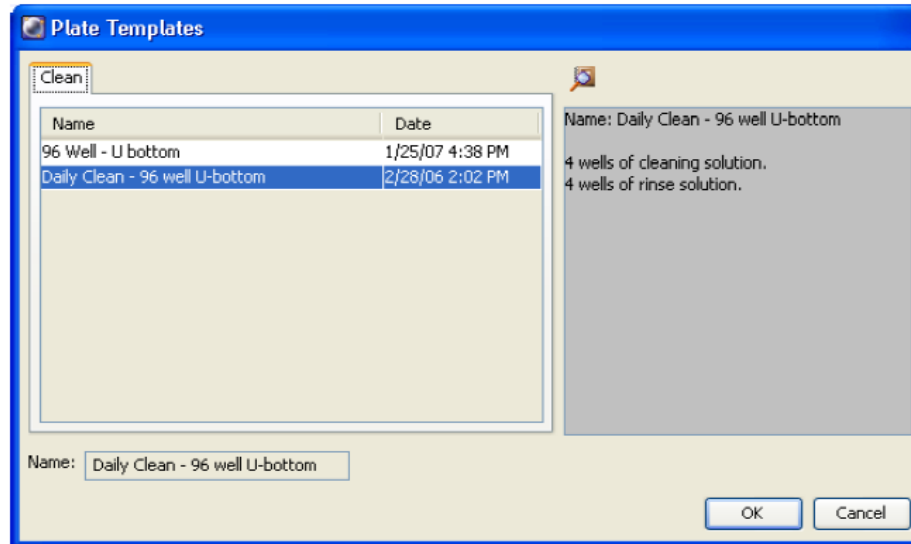


Before starting your daily workflow, ensure that your lab's software administrator has performed all the necessary tasks to set up the software for your use. This guide shows a workflow that uses application settings.

- 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.

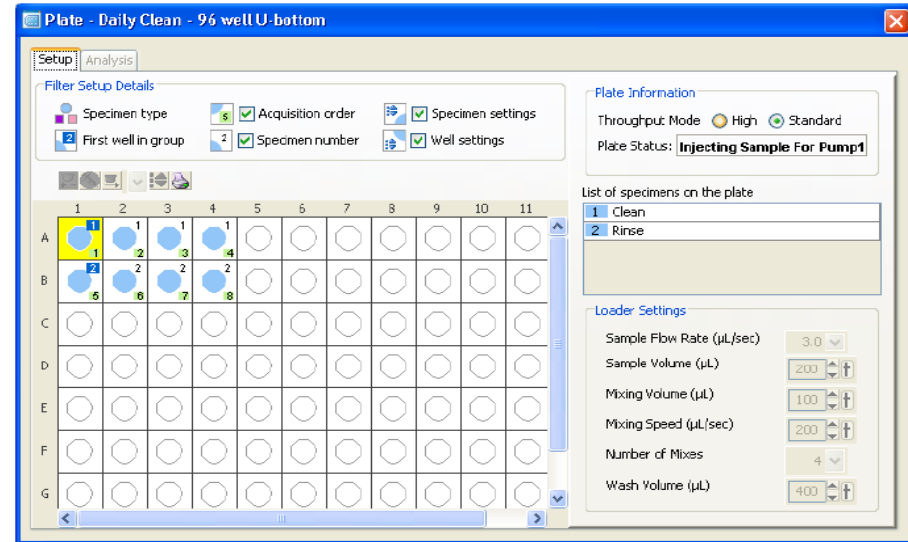
- 3 Click OK.

- 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.

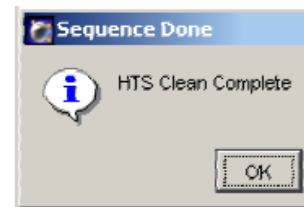
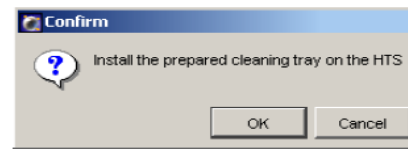
Wells	Solution	Volume ( $\mu\text{L}$ )
A1–A4	BD FACSClean <sup>a</sup>	200
B1–B4	DI water	200

a. or a 10% bleach solution

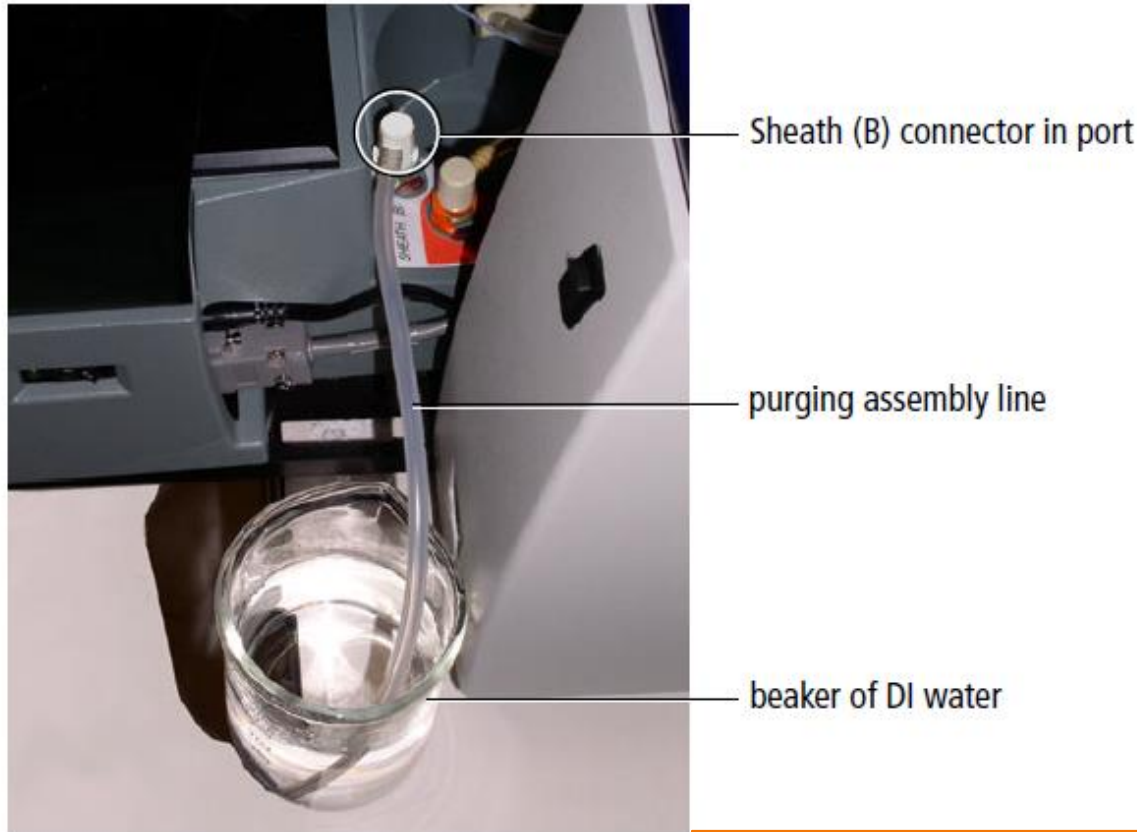


- The following message appears.

**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.