BD Accuri™ C6 Plus System Quick Reference Guide

This guide contains instructions for a daily workflow using the BD Accuri C6 Plus system with or without the BD CSampler™ Plus accessory.

Workflow Overview

The following figure shows the daily flow cytometry workflow when using BD Accuri C6 Plus system.

Start up system ▶ Instrument QC ▶ Collect data ▶ Perform a SIP clean ▶ Analyze data ▶ Shut down system

Starting Up the System

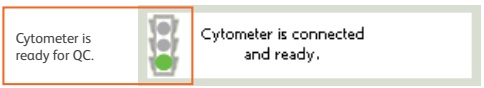
Routine startup takes approximately 13 minutes once initiated.

Check fluids

1. Check the in-line sheath filter to verify that it has not dried out.
2. Fill each bottle with the appropriate fluid:
   - 2 liters of 0.2-µm filtered deionized (DI) water with BD™ Sheath Additive to the sheath bottle
   - 250 mL of BD™ FACSClean solution to the BD FACSClean bottle
   - 250 mL of appropriately diluted BD™ Detergent Solution Concentrate to the detergent solution bottle
3. Empty the waste bottle and add 200 mL of undiluted bleach.

For manual start up:

1. Place a tube containing at least 2 mL of DI water on the SIP.
2. Press the power button on the computer system and on the front of the cytometer.



Start up system using the BD CSampler Plus

1. Press the power button on the computer system and on the front of the cytometer.
2. Click Eject Plate and load fresh cleaning tubes on the BD CSampler Plus tray.
   - 2 mL of BD FACSClean solution in the triangle (△)
   - 2 mL of DI water in the circle (○)
   - 2 mL of DI water in the square (□)

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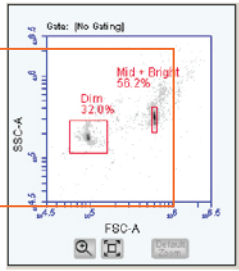
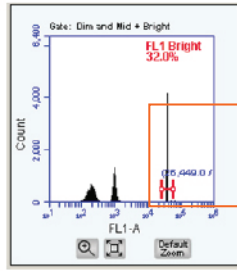
Performing Instrument QC

1. Prepare BD™ CS&T RUO beads.
2. Click the Instrument QC button.
3. Select the bead lot file from the **BD CS&T Bead Lot** menu or install a new bead lot file.
4. Load the CS&T RUO bead tube with the correct lot number.
5. Click RUN.

Adjust the regions to encompass the dim and mid + bright beads.





Adjust the bright red peak markers.

6. Analyze the results.

Parameter	Bright Bead Median	MFI Range		% Bright Bead rCV	Instrument Sensitivity	Sensitivity Spec.	Parameter Pass/Fail
FSC	588246	412275	765654	1.7%	198	30	Pass
SSC	312137	215590	400381	11.2%	73	50	Pass
FL1	36529	24614	45711	2.2%	272	80	Pass
FL2	34382	23474	43594	2.3%	585	200	Pass
FL3	71922	48908	90829	3.5%	72	40	Pass
FL4	56124	42080	78149	4.9%	143	70	Pass

Check whether the parameters have passed, then click **Print**.

7. Close the Instrument QC workspace.

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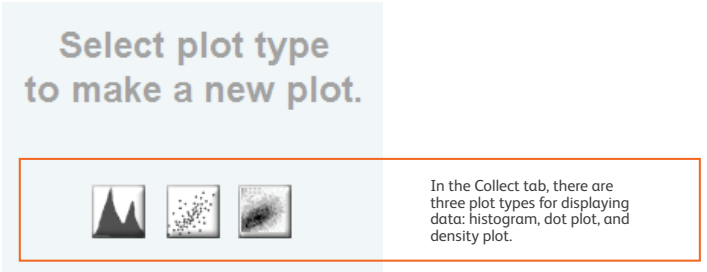
Collecting Data

For information on collecting data with the BD CSampler Plus, see the *BD Accuri C6 Plus System User's Guide*.

Prepare the workspace

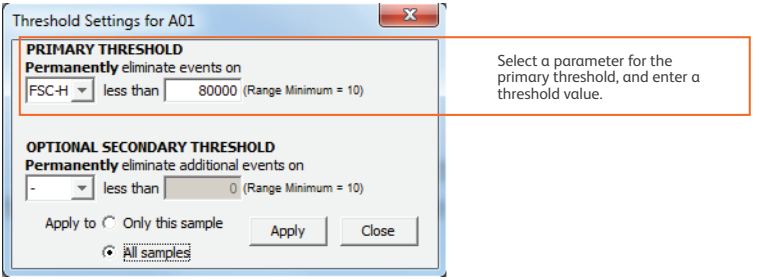
1. In the Collect tab, enter sample names in the sample naming field.
2. Create plots.



3. If desired, rename the plot parameters.

Set the threshold

1. Use the zoom ( ) and unzoom ( ) tools to ensure that the population(s) of interest is clearly displayed.
2. Click Set Threshold.



3. Click Apply.

Set color compensation

1. For multicolor experiments, apply compensation by clicking Set Color Compensation.
2. Click **Preferences**. Select your preferences, and click **Save**.

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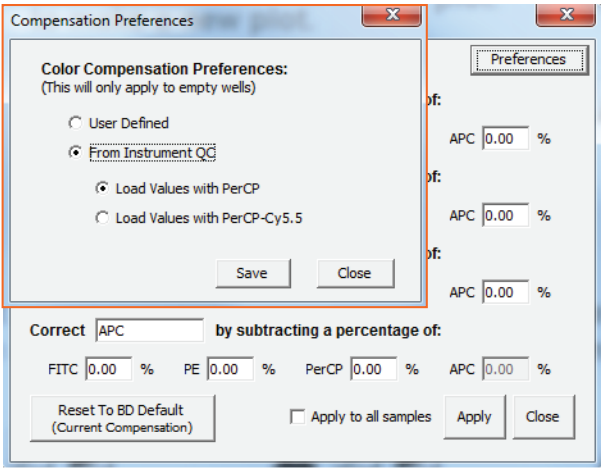
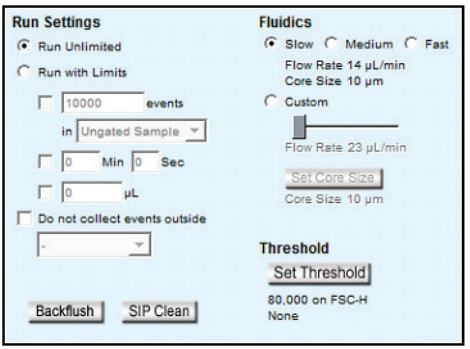


NOTE The compensation values from the instrument QC are only valid if you are using the identical fluorochromes in your experiment: FITC, PE, PerCP or PerCP-Cy™5.5, or APC. If you are using a different combination of fluorochromes, then you should select **User Defined** and run the appropriate compensation control tubes to calculate compensation.

Set the Acquisition Criteria and Collect Data

❶ Under **Run Settings**, select acquisition criteria for automatically stopping data collection. Draw a gate on a plot if you wish to set a stopping criterion on gated events.



❷ Under **Fluidics**, select the flow rate.

❸ Select a position in the sample grid, load the corresponding tube, and click RUN.

Performing a SIP Clean Manually

Run after collecting samples, and if the instrument is left idle for 15 or more minutes.

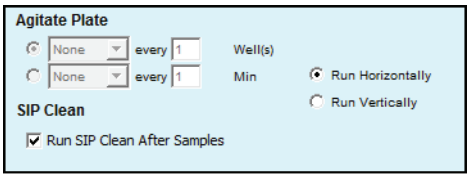
1. From the Collect tab, click SIP Clean.
2. A dialog will open prompting you to load a tube containing 2 mL of BD FACSClean solution.
3. Load the tube of BD FACSClean solution and click **SIP Clean**.
4. When step 3 is complete, a dialog will prompt you to load a tube containing 2 mL of water.
5. Load the tube of water and click **SIP Clean**.

Performing a SIP Clean Using the BD CSampler Plus

Run after each plate, and if the instrument is left idle for 15 or more minutes.

From the Auto Collect tab:

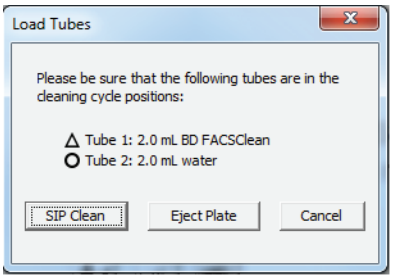
1. Before collecting data, ensure the appropriate cleaning tubes are in the designated locations on the BD CSampler Plus.
2. Verify that the **Run SIP Clean After Samples** checkbox is selected.



The BD CSampler Plus will automatically run a SIP clean after all of the samples in the plate have been collected.

From the Manual Collect tab:

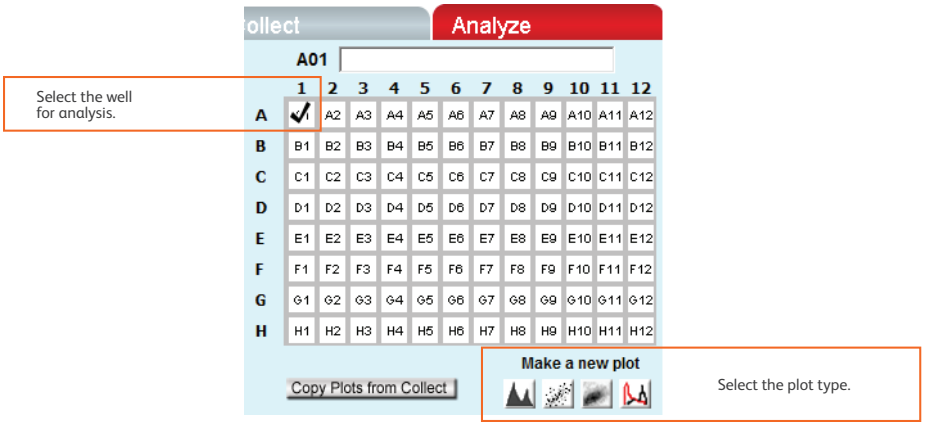
1. Click SIP Clean in the **Manual Collect** tab.
2. Ensure the appropriate cleaning tubes are in the designated locations on the BD CSampler Plus.



3. Verify that the square (☐) location also has a tube containing 2 mL of DI water.
4. Click **SIP Clean**.

Analyzing Data

1. In the Analyze tab, create plots and gates needed for analysis.



2. To print plots and their associated statistics:
   a. Select the checkbox in the upper-left corner of each plot that you want to print.
   b. Select **File > Print Selected Items**.

3. To export data, do one of the following:
   - Select **File > Export FCS File** to export and save the data from the currently selected well as an FCS 3.1 file. Enter a file name and click **Save**.
   - Select **File > Export ALL Samples as FCS** to export and save the data for all wells as individual FCS 3.1 files. A default folder is created on the desktop. Click **Ok**.
   - Select **File > Export Plot Data as CSV** to save an individual file in CSV format with the information for each event in the well. Enter a file name and click **Save**.
   - Select **File > Export Sample Settings** to export acquisition criteria, sample names, parameter names, and compensation values to a CSV file. This option is available only for BD CSampler™ Plus software.

Shutting Down the System

Shutdown takes approximately 13 minutes once initiated.

Manually

1. Place a tube with 2 mL of DI water on the SIP.
2. Press the power button on the cytometer to turn it off.
3. Shut down the computer.

Using the BD CSampler Plus

1. Ensure fresh cleaning tubes are on the BD CSampler Plus, and press the power button on the cytometer to turn it off.
2. Shut down the computer.

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