

Training for Accuri C6 Plus

❖ Accuri-Specific Notes:

- Fixed Voltages– The accuri PMTs have a fixed amplification applied, meaning that there are no voltage adjustments unlike traditional systems. This is accomplished by having a 7 decade dynamic range as opposed to the standard 5 decade range.
- The wide dynamic in the system means that the data may need to be rescaled during data analysis. If you have trouble with this please ask the Flow Core staff.
- The Accuri is a volumetric injection system allowing for accurate information on volume collected and thereby allowing for cell count and concentration calculations. This also allows for many different types of tubes to be used on the system.
- **Digital Compensation- The Accuri C6 Plus software does not come with an automatic digital compensation program. Our recommendation for all users is to run their samples uncompensated and complete the compensation offline in FCS Express or Flowjo. Questions on this topic can be directed to the Flow Core staff.**

Basic Procedure:

1. Log in to PPMS
 2. Check the fluids (sheath, waste, cleaning fluids)
 3. Open the software
 4. Start the instrument (if you are the first user)
 5. Backflush and run water – Save Workspace file at start of water run.
 6. Set run limits and fluidics control
 7. Create Plots – Label parameters
 8. Run wells – Save Workspace file intermittently
 9. Run Daily Clean – Leave water tube on the SIP
 10. Export FCS files – Rename FCS files on hard drive
 11. Close Software – Save Workspace file during close
 12. Check the fluids (sheath, waste, cleaning fluids)
 13. Check calendar – Shut down instrument if you're the last user
 14. Log out of PPMS
- **Start Up:** Check the fluid bottles on the side of the machine **BEFORE** hitting the power button. Ensure that the sheath is not empty and the waste is not full, and that the decon and detergent bottles are full. If you are the first user for the day, turn the cytometer on by pressing the power button on the front of the instrument. (The light above the power button should flash blue and the pumps should turn on)
 - ***Warning* If the sheath is empty and/or the waste is full, both blue lights on the front of the instrument will blink. If this happens, the instrument will not start. Hold the power button down until the machine powers off. Make sure the sheath is full and the waste is empty, then power the instrument back on. After this happens, startup will take approximately 25 minutes due to the shutdown override.**
 - Wait approximately 15 minutes for pumps to finish cycling (normal startup)
 - Sheath: dH₂O can be found at most lab sinks
 - Decontamination: Additional solution can be found near the instrument. (*Do NOT use the regular bleach*)
 - Detergent/Cleaning: Additional solution can be found near the instrument.
 - Screw Caps on Fluid bottles making sure the caps match the bottles.
 - **Log into PPMS**
 - At the completion of the training the new user will have their access approved.
 - **Start BD Accuri C6 Plus Software**
 - Double click the BD Accuri C6 Plus icon to start the software.
 - Confirm that the Cytometer and Computer are connected using the status light in the software.

- **Perform a Quick Clean:** process is posted at the instrument.
 - Put the backflush tube on the SIP and press backflush in CFlow Software.
 - Put a fresh tube of water on the SIP and let it run on high for 2 minutes.

- **Collecting data in BD Accuri C6 Plus:** Samples (and controls) can be collected in any position on the “Plate” in the software. Any settings will be remembered between samples until they are changed by the user.
 - Set Run Limits- set the number of events to collect (in total or in a specific gate), or set the time to collect, or set the volume to collect. If more than one setting is activated, the first one reached will be the limiting factor.
 - Set fluidics- The slower the run speed the more sensitive the measurements made by the instrument. Increasing run speed will increase coincidence events and spread of the fluorescent signals.
 - Label the parameters by clicking on plot axis and then on “Rename Parameters”. Be sure to activate the new name for all samples.
 - Plots and gates can be drawn while running, but are not required to record data. Most data analysis will be done offline.

- **Exporting and Saving your data:** Data Management is explained in more detail in the document posted at the instrument. Data should be saved as a Workspace file and as FCS files.
 - Workspace files are similar to “experiment” files on a diva instrument. They are software specific and can’t be opened in offline analysis programs. When you first begin running your experiment you will be prompted to save a workspace file. This file should be saved in the existing folder marked with the current year and month.
 - At the completion of your experiment you should export your files as FCS to be opened for data analysis in offline analysis software. Open the file menu and click on Export all files as FCS. The FCS files will be exported to the FCS Exports folder located on the computer desktop. You can rename the FCS file folder on the hard drive.
 - Transfer your FCS files to the transfer pipeline to access files back in the lab. Open the Shortcut to Transfer from the desktop and drag and drop your FCS files into your PI’s folder.
 - Your workspace file will save automatically as you progress, but it’s never a bad idea to save the file at the completion of your run when shutting down the Accuri C6 Plus software.

- **Shut Down:** The cytometer must be shut down if you are the last person scheduled on the calendar.
 - Perform a Daily Clean – the procedure is posted on the instrument. Be sure to leave the water tube on the SIP upon completion.
 - Exit out of BD Accuri C6 Plus Software.
 - Check the Sheath, Waste, Decon, and Detergent bottles on the side of the machine. Refill them if needed and empty the waste if the bottle is $\frac{3}{4}$ full or more.
 - Check the calendar when you finish (make sure to refresh the page) to see if anyone else has signed up.
 - If no one is signed up add a clean tube of water to the SIP and press the power button – the system will begin its cleaning process and shut itself down automatically. ***Note: Do not hold the power button for too long or you will override the shutdown procedure, resulting in a prolonged startup time the next time the Accuri is turned on.***
 - Log out of PPMS by clicking “Logout”.

- **Troubleshooting:**
 - Most issues on the Accuri can be traced to a dirty instrument:
 - Run a **new clean** water tube for 5 minutes on high. The events/sec should be ~15 or less.
 - Run an additional daily clean cycle, or a full shut down and start up to reset fluidics.
 - Run a “Clean Fluidics” cycle under the “Instrument” tab in the software.
 - Run a “SIP clean.” The button is located in the software on the left side, below the Run Settings.
 - Run multiple backflushes to clear minor clogs:
 - If no fluid is flushed out, run decon and clean solution on high and backflush again. If the problem persists place the water back on the SIP and shut down.

- **Instrument Issues:**
 - If you cannot fix an issue you should immediately log the issue in the **Instrument Tracking Log**. The tracking log is one of the home pages in Chrome and directions are posted on the instrument. This will send an email to all members of the Flow Cytometry Resource staff explaining the situation.
 - The more information you provide the easier it is to diagnose the problem.
 - You should also write a brief note to any potential users after you explaining that there is an issue.

Additional CFlow Software information can be found in the CFlow Users Guide located in the URMC Flow Core website library.

BD Accuri™ C6 Plus

Instrument QC



Collect

Analyze

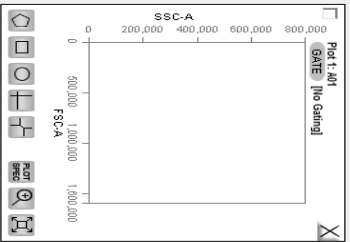
Statistics

Batch Analysis

A01

	1	2	3	4	5	6	7	8	9	10	11	12
A	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12
B	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12
C	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12
D	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
E	E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12
F	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
G	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12
H	H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12

Chrometer not connected.



Select plot type
to make a new plot.

Select plot type
to make a new plot.

Select plot type
to make a new plot.

Select plot type
to make a new plot.



Run Settings

Run Unlimited

Run with Limits

10000 events

In Ungated Sample

0 Min 0 Sec

0 μ L

Do not collect events outside

Threshold

Set Threshold

80,000 on FSC-H

None

Backlist

SIP Clean

RUN

Fluidics

Flow Rate - μ L/min

Core Size - μ m

Custom

Flow Rate - μ L/min

Core Size - μ m

Set Core Size

Do not collect events outside

Threshold

Set Threshold

80,000 on FSC-H

None

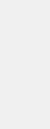
Backlist

SIP Clean

RUN

Select plot type
to make a new plot.

Select plot type
to make a new plot.



Plot (A01)	Count	Volume (μ L)	% of This Plot	% of All	Mean FSC-A	Mean SSC-A	CV FSC-A	CV SSC-A	Median FSC-A	Median SSC-A
All										

Set Color Compensation

Last Run

Events

0

Time

0:00:0

Microcolliers

0

Events / Sec

0

Events / μ L

0

Data Capacity Used

0%

of 98,000,000 Events