Accuri Student Use Cytometer – Quick Guide

Start-up (if the machine is off)

1. Check water on SIP = 2 ml or more.
2. Push instrument power button & wait (~6 min).
3. Run water on Fast, 10 min.

Run

1. Vortex & mount the sample. Secure the pedestal.
2. Set Limits, including the amount of µl's at less volume than your sample.
3. Pick where to store the data in the grid and click RUN.
4. Watch the real-time reports on screen and stop if there are issues:
   Event rate should be between 400-1000 events/second
   Abnormal patterns, pauses, or rapid drops in event rates indicate a problem
5. Remove the sample.
6. Wipe down the SIP with a kim wipe.
7. Optional: run a sample of your media for 1 second between samples to prevent carryover

Flush (required once per hour or if you will leave the machine on)

1. Click "SIP Clean" and follow the instructions
2. If the flush is to leave the Accuri on for another user:
   a. Leave a note to say when you finished the flush.
   b. Close the program and log out of Windows.

Shutdown

1. Click "SIP Clean" and follow the instructions
2. Set Filtered water on SIP = 2 ml or more.
3. Push instrument power button for auto shutdown.
4. Close the program and log out of Windows.

Channels

FSC = Forward Scatter
SSC = Side Scatter
FL1 = FITC, GFP, etc.
FL2 = PE, PI, etc.
FL3 = PerCP-Cy5.5, PECy7, etc.
FL4 = APC, APC-Cy7, etc.

Compensation

1. Click "Set Color Compensation"
2. Click on "Preferences" in the top right hand corner
3. Select "From instrument QC" and select load from PerCP or PerCP-Cy5.5 depending on the color you are using (or leave as PerCP default if you are not using this channel)
4. Select "Save"

If you want to do a customized compensation, please talk to Dr. Hope to receive training.

Laser	Fluorescent Channel	Channel Names / Dyes
488 nm – Blue	533/30	FL1, FITC, GFP, Alexa-488, SYBR Green, etc.
	585/40	FL2, PE, PI, etc.
	670 long pass	FL3, PerCP-Cy5.5, PE-Cy7, mCherry, DsRed, etc.
640 nm – Red	675/25	FL4, APC, Alexa-647, etc.

Student Use BD Accuri Flow Cytometer

1. Training Requirements: Before you are approved to use the Accuri student-use cytometer, you must take the first-hour informational training, the second-hour equipment training, and watch the two Accuri training videos posted on the RIC website. You must also demonstrate the ability to load and unload a sample.

2. Computer Access: Log in is by BYU Net ID and only approved users have active accounts. Complete all of your training requirements and then request the RIC lab technician to submit your NetID for activation.

3. RIC Access: You must have your professor email a request for RIC door access and LSB building access to the LS Safety Officer, Rebecca Scholl, rebecca_scholl@byu.edu , for your BYU ID card to be activated. The RIC door is only open when technicians are present. Building access is required for any after-hours.

4. Use the Scheduler: Please be sure to use the online scheduler for "Accuri Flow Cytometer". Appointments are open Mon-Sat, 24 hrs and you can add appointments anytime (even the day-of, unlike the large cytometer which requires sign-up in advance for technician-run samples). The scheduler reserves an hour, to account for Startup and Shutdown in addition to time for running samples. https://ricfacility.byu.edu Please delete appointments you won't keep!!

5. Prepare your Samples: The cytometer fluidics has an opening of 70 um for samples to flow through for analysis. The cytometer is subject to clogging if your cells clump or if you try to run large items or large cells. We highly recommend using the 70 um mesh filter (cell strainer) for all samples prior to running. Your professor can buy cell strainers or nylon mesh fabric. We keep some strainers on-hand for sale in the RIC but we don't want to be your main supplier, but rather a back-up if you are out.

6. Run your Samples: Samples should be vortexed immediately before running. The vortexer should always be on the bench not attached to the main bay bench since vibration can negatively affect the instruments. Set limits on your samples to tell the Accuri how many events to count, how long to run, or how many ul's to run. Always set limits on ul's of sample to less than your total volume to avoid drawing up air. Make sure to keep the events per second under 1,000. Between samples, use a KimWipe to wipe down the sip. If backwashing is occurring between samples (particularly bacteria), use a sample of media between samples to reduce contamination and run for 1 sec.

7. Save Data Files: Exporting files includes several formats such as Workspaces, Templates, and FCS files. You are responsible for saving your own data files on another device or computer.

8. Shutdown or Flush the Machine: If someone will be using the machine immediately after your appointment, run a FLUSH and leave a note. If no one is using the machine after you, just run Shutdown.

Note: You must finish the entire SIP clean (Facs and water) before exiting the software.

9. Exit Software and Log Off: Once the SIP is completed, make sure that you exit out of the software. After you have closed the software, make sure to log out of the computer.

10. Report your Usage: Please fill out usage cards based on time that you run your actual samples and drop the card in the box on the way out of the RIC. DO NOT include start-up time, shut-down time, or the hourly FLUSH time in your usage time. We do not want your professor to pay for these, only for the time to run your samples.

11. Solve problems: The lab technicians are the first line of help. If you have a problem and the technician is not in the RIC, there is a manual for the equipment in the drawer beside the machine. You can also contact one of the technicians and use the RIC phone near the technician desk to contact the technicians or to call someone's cell (see below).

12. Get Help: Please email or ask Dr. Hope or the RIC technician any questions you might have!

RIC technicians: Jordan (801)739-4077, Maddie (801)837-0695.
RIC phone: 801-422-3195 Dr. Hope's cell: (801)860-1681
RIC email: ric_tech@byu.edu Dr. Hope email: sandrahope2016@gmail.com