

abcam

BD Accuri C6 with single tube loader protocol

For multiplex assays

This protocol contains instructions for setting up an Accuri C6 with Tube Loader. If you will be using a different system, consult the appropriate protocol in our flow cytometry protocols for multiplex assays page, or contact our Technical Support team at technical@abcam.com.

For first time use of a system, please follow the cytometer setup/verification. After a system is properly validated, those settings can be saved and used for routine assay readout with the method for assay readout.

Please read this protocol carefully before cytometer operation. Failure to properly set up a system may result in unusable data, loss of product or system damage. For more detailed instructions on system operation details. Please consult the Accuri C6 operation manual.

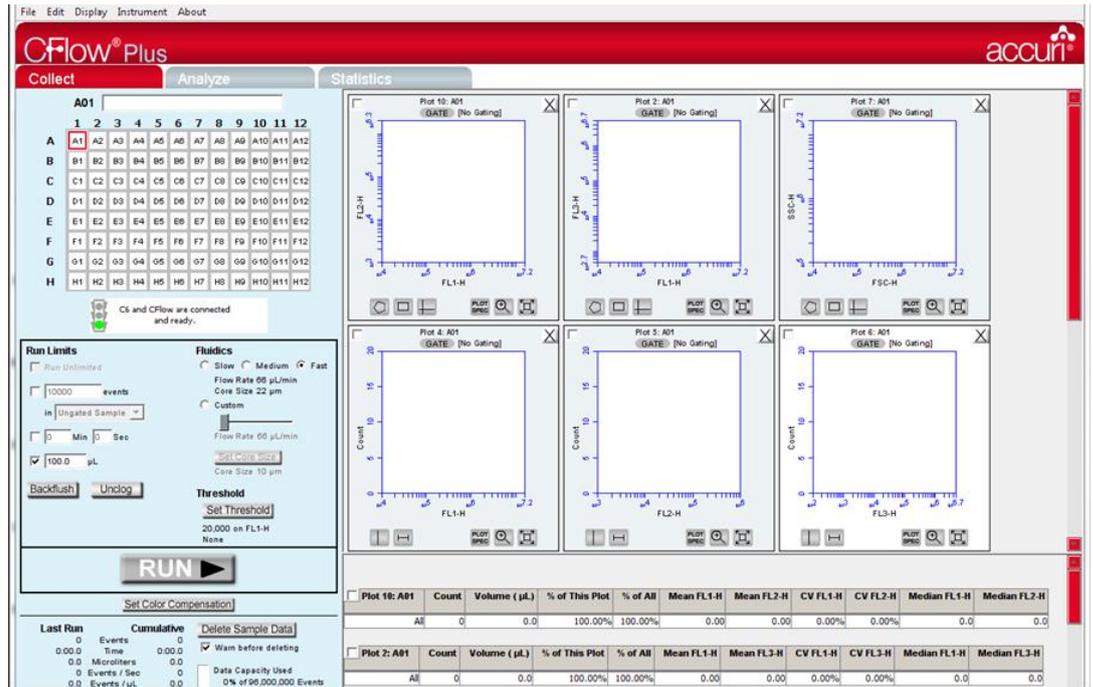
Requirements

- Multiplex Immunoassay Core Kit OR miRNA Assay Core Reagent Kit
- Pipette and tips
- Tungsten cleaning wire (only if system SIP is obstructed)

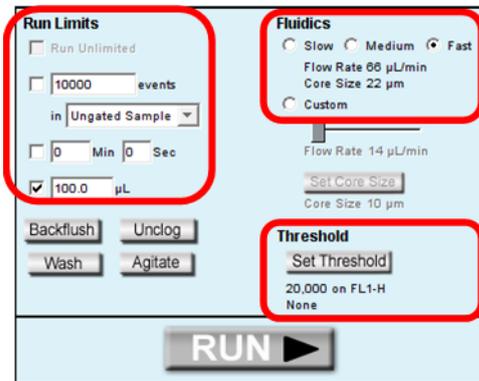
Cytometer setup/verification (first time use only)

1. If using a Multiplex Immunoassay Core Kit follow the below instructions:
 - a) Prepare 1x Capture Particle Master Mix as per step 9.6 in the Human Multiplex Immunoassay Core Kit instructions.
 - b) Invert the 1x Capture Particle Master Mix five times to mix. Add 75µL of 1x Capture Particles to a 1.5mL Eppendorf tube.
 - c) Add 100µL of Run Buffer to the well and vortex briefly to resuspend the particles.
2. If using a miRNA kit follow the below instructions:
 - a) Invert the Firefly™ Particles five times to mix. Add 35µL of Firefly™ Particles to a 1.5mL Eppendorf tube.
 - b) Add 140µL of Run Buffer to the well and mix using aspiration to resuspend the particles.
3. Download the Accuri Tube Loader template file from www.abcam.com/FireflyCytometry.
4. Start up the Accuri System and open the CFlow Plus Software. Be sure to perform all requisite system startup and cleaning functions.

- Under the **"File Menu"**, select **"Open CFlow File or Template"**. Select the Accuri template file to load. Once the template has loaded, six plots should be displayed on your screen as illustrated below.



- Locate the Acquisition Control section located in the middle-left section of the screen. Ensure that the following settings are selected:
 - Threshold is set to "20,000 on FL1-H".
 - Run Limits is set to "100 μ L".
 - Fluidics is set to "Fast".

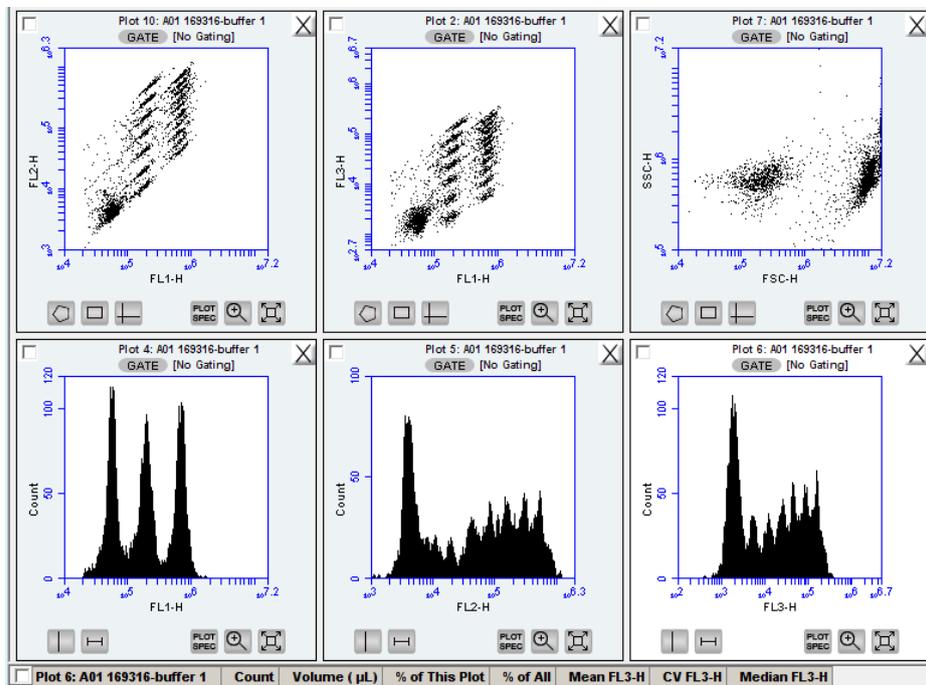


Failure to load the correct settings file will lead to loss of data.

- Place a Kimwipe under the Sample Injection Port (SIP) tube and click on the "Backflush" button. Observe flow out of the SIP tube. The flow from the instrument should briefly be a steady and continuous stream for part of the procedure versus a closely spaced set of distinct droplets. The flow will only be a stream for around one second of the backflush if the instrument is working properly.
 - If the flow is a continuous drip without a period of steady flow insert a tungsten cleaning wire (VWR 72310-068) approximately 2 inches into the SIP to clean the flow cell. Repeat the backflush to see if the flow has improved to a steady stream.
 - If performance does not improve after cleaning the flow cell, ensure peristaltic pump tubing has been changed as per manufacturer recommendations.

Proceeding without a properly flowing instrument will yield poorly resolved codes with potential data loss.

8. Vortex the sample and place it on the SIP tube. Click "Run" button located under the Acquisition Control section. When prompted, save the .c6 file to a hard drive location and the data acquisition will begin. Data acquisition should appear similar to the image below.



9. After the tube has been acquired, you must export the data as an FCS file to be able to analyze in the Firefly™ Analysis Workbench. Select the well, then go to "File Menu" and select "Export All Files as FCS". Locate the FCS file and transfer it to a USB drive to import it to your personal computer.
10. Download the Firefly™ Analysis Workbench onto your personal computer from <http://www.abcam.com/kits/firefly-analysis-workbench-software-for-multiplex-mirna-assays>. Reference your Core Kit instructions for detailed instructions on how to use the Analysis Workbench software.
11. Open the Firefly™ Analysis Workbench and load the FCS file saved in Step 11. When prompted, load the PLX file for your particle mix. Once the data loads, check the color of the well, a Green well indicates a successful setup.

If the quality indicator is Red, perform a thorough system clean, and repeat this protocol. If the score remains low, contact our Technical Support team at technical@abcam.com.

Single tube collection of all samples

Important – only run this protocol after you have performed “Cytometer Setup/Verification” protocol on your BD Accuri C6 cytometer using particles. This protocol also assumes a completed assay transferred into FACS or Eppendorf tubes, with particles suspended in run buffer.

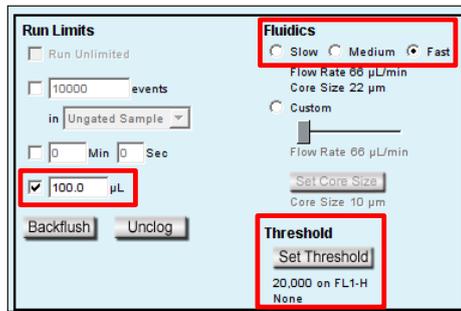
1. Start up the Accuri System and open the CFlow software. Be sure to perform all requisite system startup and cleaning functions.
2. Under the “**File Menu**”, select “**Open CFlow File or Template**”. Select the Accuri template file to load. Once the template has loaded, six plots should be displayed on your screen as illustrated in Step 5 of the Cytometer Setup/Verification protocol.

Failure to load the correct settings file will lead to loss of data.

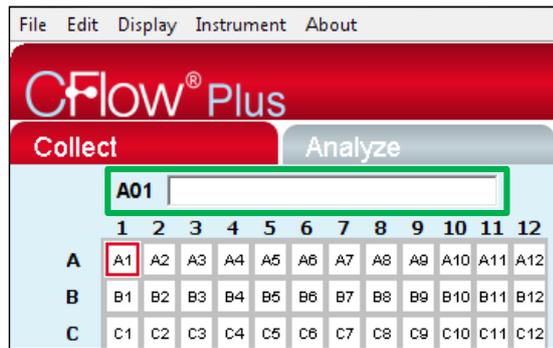
3. Place a Kimwipe under the Sample Injection Port (SIP) tube and click on the “Backflush” button. Observe flow out of the SIP tube. The flow from the instrument should briefly be a steady and continuous stream for part of the procedure versus a closely spaced set of distinct droplets. The flow will only be a stream for around one second of the backflush if the instrument is working properly.
 - a. If the flow is a continuous drip without a period of steady flow insert a tungsten cleaning wire (VWR 72310-068) approximately 2 inches into the SIP to clean the flow cell. Repeat the backflush to see if the flow has improved to a steady stream.
 - b. If performance does not improve after cleaning the flow cell, ensure peristaltic pump tubing has been changed as per manufacturer recommendations.

Proceeding without a properly flowing instrument will yield poorly resolved codes with potential data loss.

4. Vortex the sample and place it on the SIP tube. Click the corresponding well from the filter plate in the grid to select it and identify the origin of the sample.
5. Locate the Acquisition Control section located in the middle-left section of the screen. Ensure that the following settings are selected:
 - a) **Threshold** is set to “20,000 on FL1-H”.
 - b) **Run Limits** is set to “100 µl”.
 - c) **Fluidics** is set to “Fast”.



- Optional: Name the individual samples you will acquire using the Text box above the 96-well plate graphic.



- Click the "Run" button located beneath the Acquisition Controls section. The program will prompt you to select a name and location for the *.c6 file you will create. Save your *.c6 experiment file to the hard drive and sample acquisition will commence.
- After the sample has finished scanning, repeat starting at step 4 with the next sample.
- Once all the samples have been acquired, go to "File Menu" and select "Export All Files as FCS". Locate the FCS files and transfer them to a USB drive to import it to your personal computer. Load the exported FCS file into the Firefly™ Analysis Workbench for analysis.

