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BD Accuri C6 with plate handler

Protocol for multiplex assays

April 2016

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This protocol contains instructions for setting up an Accuri C6 with plate handler. 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 Multiplex miRNA Assay Core Reagent Kit
- Blade (scalpel or scissors)
- 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:

    - Prepare a 1x Capture Particle Master Mix according to the Multiplex Immunoassay Core Kit Booklet.
    - Cut and remove the plate seal from well H12 on the provided filter plate.
    - Add 75µL of 1x Capture Particle Master Mix to the well, and apply vacuum using a vacuum manifold to remove the buffer from the well.
    - Add 100µL of Run Buffer to the well.

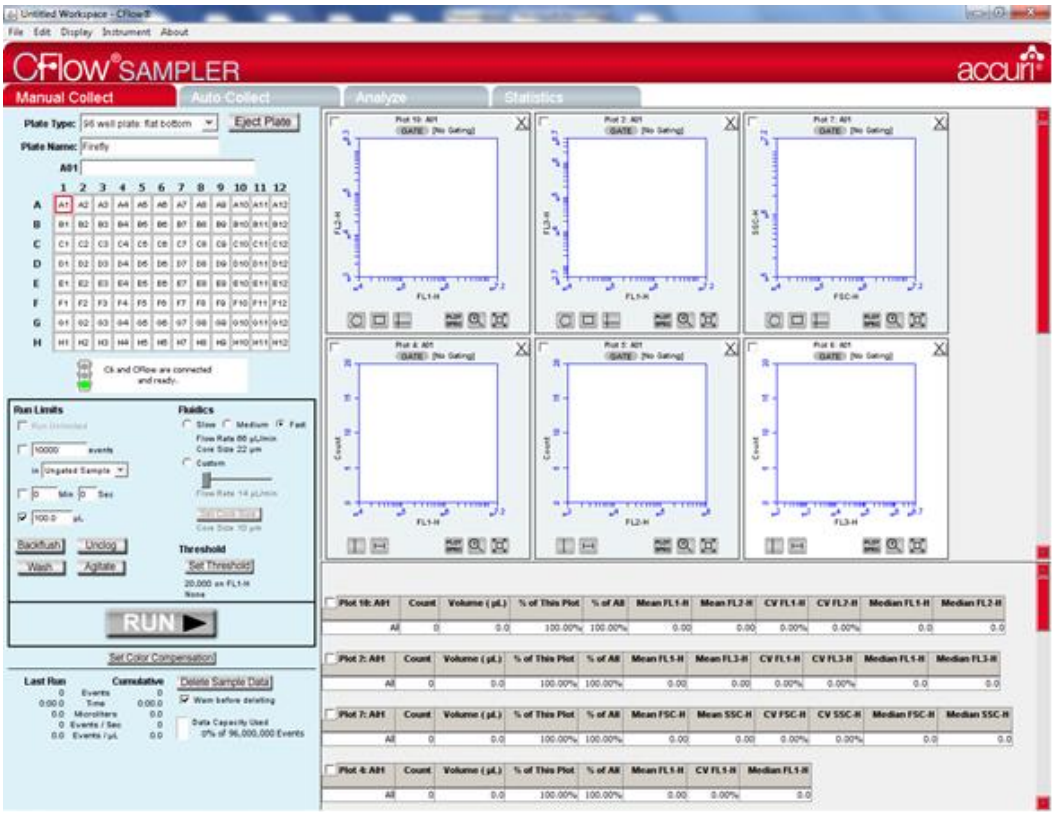
2. If using a miRNA Core Reagent Kit follow the below instructions:

    - Cut/remove the plate seal from well H12 on the provided filter plate.
    - Add 35µL of Firefly™ Particles to the well, and apply vacuum to remove all liquid from the well.
    - Add 140µL of Run Buffer to the well

3. Download the Accuri Plate template file from www.abcam.com/FireflyCytometry.

4. Start up the Accuri System and open the CFlow Software. Be sure to perform all requisite system startup and cleaning functions for the cytometer.

5. 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.



6. 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".

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

7. Click the "**Eject Plate**" button in the top left corner of the control screen to load the plate.

   Touching the plate handler before it has stopped moving may cause the arm to require recalibration.

8. With the arm still in the ejected position, 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.

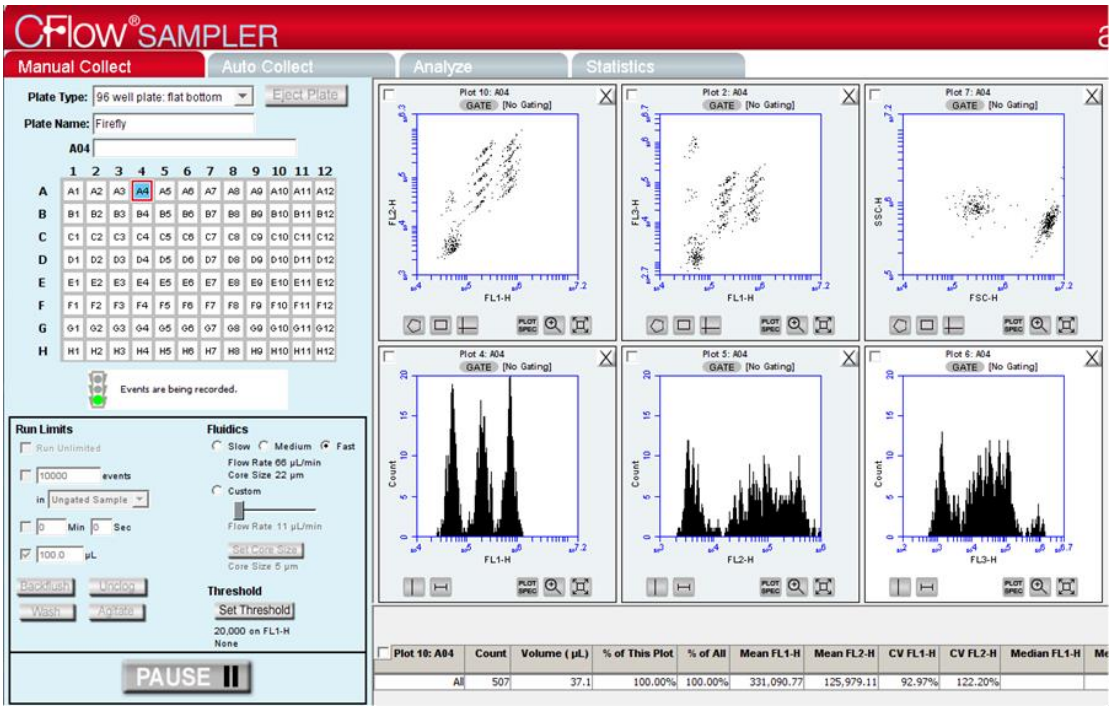
   a) If the flow is a continuous drip without a period of steady flow insert a tungsten cleaning wire (VWR 72310-068) approximately 2 inches into the SIP to clean the flow cell. Repeat the backflush to see if the flow has improved to a steady stream.

   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.

9. Load the plate in the handling arm, aligning the "**A1**" well of your plate with the indicated position on the plate platform. Click the "**Load Plate**" button to retract the arm into the instrument.

10. Select the well in the plate grid and click the "**Run**" button directly below the Acquisition Control panel. 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.



11. After the well 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.

12. 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.

13. 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.

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Multi-well Auto Collect sample acquisition

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 in a 96-well plate, with particles suspended in Run buffer.

1. Place your plate on a shaker for one minute to resuspend the particles. After shaking, particles are stable in suspension for 30 minutes before scanning.

2. Start up the Accuri System and open the CFlow software. Be sure to perform all requisite system startup and cleaning functions.

3. 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.

4. Click the "**Eject Plate**" button in the top left corner of the control screen to load the plate.

   Touching the plate handler before it has stopped moving may cause the arm to require recalibration.

5. With the arm still in the ejected position, 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.

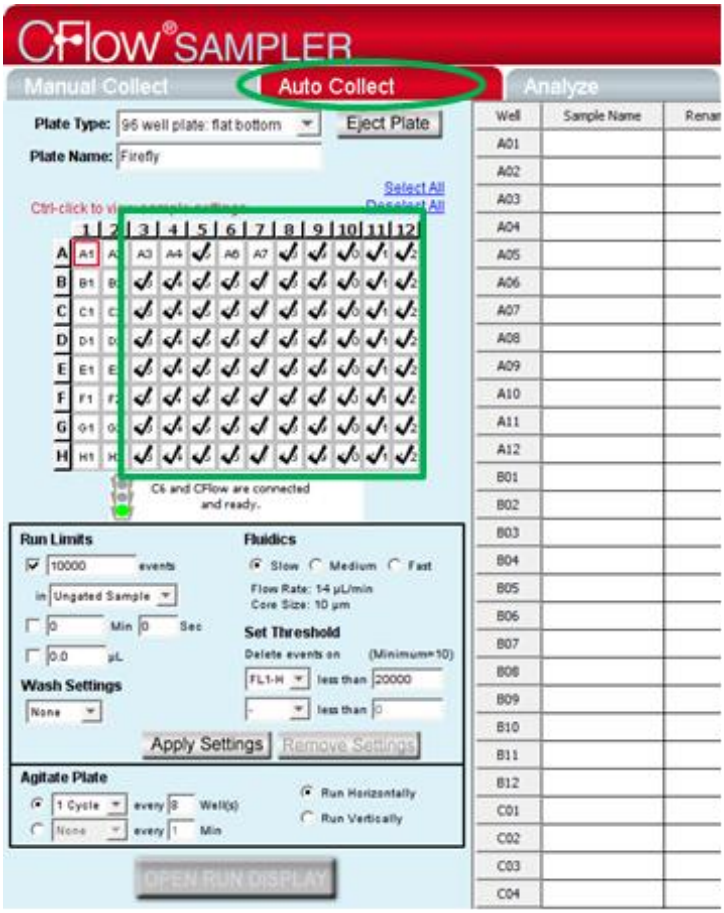
   a) If the flow is a continuous drip without a period of steady flow insert a tungsten cleaning wire (VWR 72310-068) approximately 2 inches into the SIP to clean the flow cell. Repeat the backflush to see if the flow has improved to a steady stream.

   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.

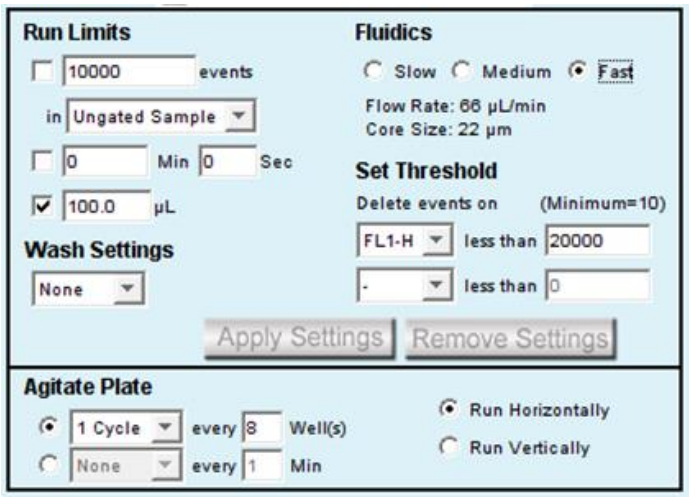
6. Load the plate in the handling arm, aligning the "**A1**" well of your plate with the indicated position on the plate platform. Click the "**Load Plate**" button to retract the arm into the instrument.

7. Click on the "**Auto Collect**" tab and select the appropriate assay wells to be acquired; selected wells will be highlighted with a check mark as illustrated below.

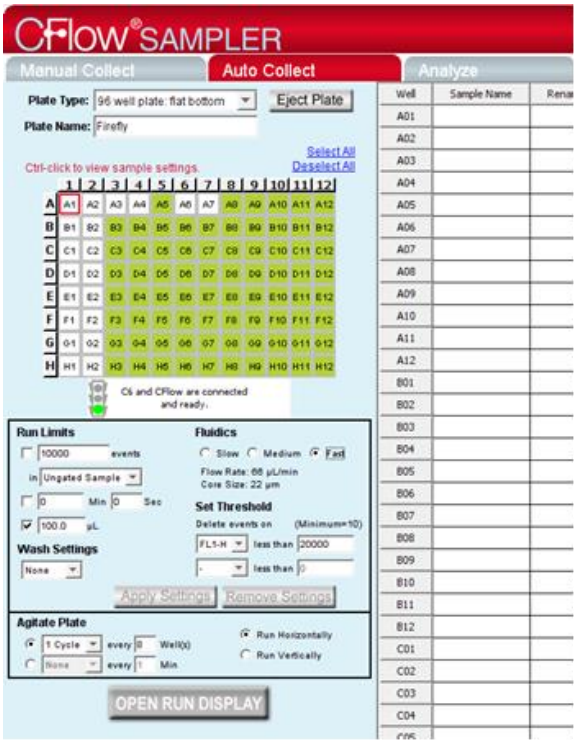


8. Locate the Acquisition Control section located in the middle-left section of the screen. Ensure that the following settings are selected:

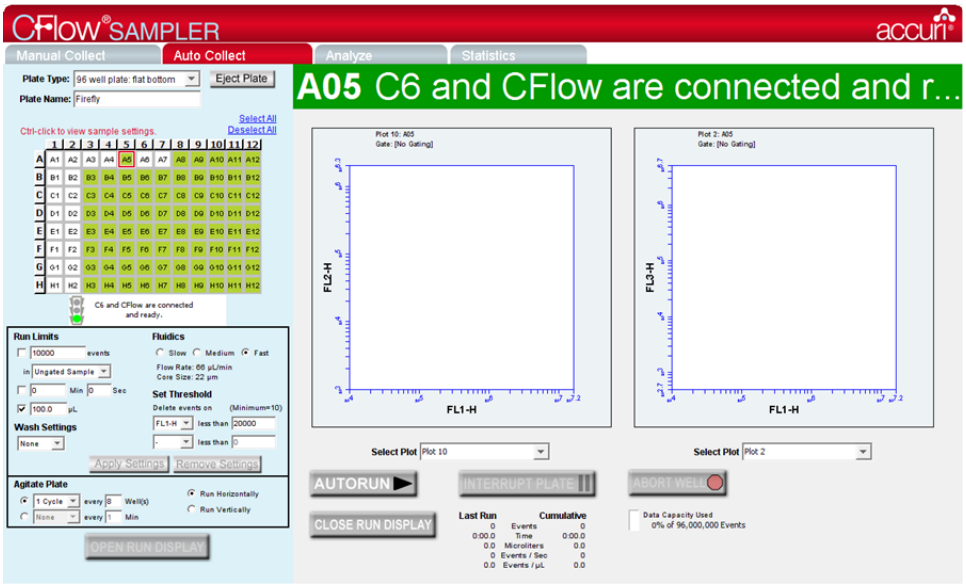
   a) **Threshold** is set to "20,000 on FL1-H".
   b) **Run Limits** is set to "100 µl".
   c) **Fluidics** is set to "Fast".
   d) **Agitate Plate** is set as "1 Cycle every 8 wells".



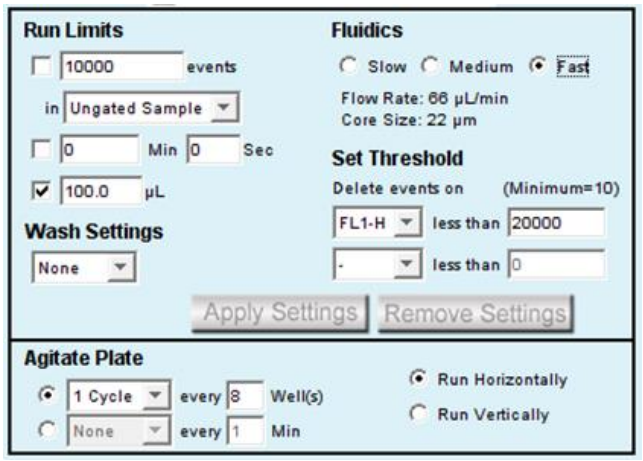
9. In the Acquisition Control section, click the "**Apply Settings**" button and then save your *.c6 experiment file to the hard drive. The check marked wells will turn Green if they have been properly selected for acquisition as illustrated below.



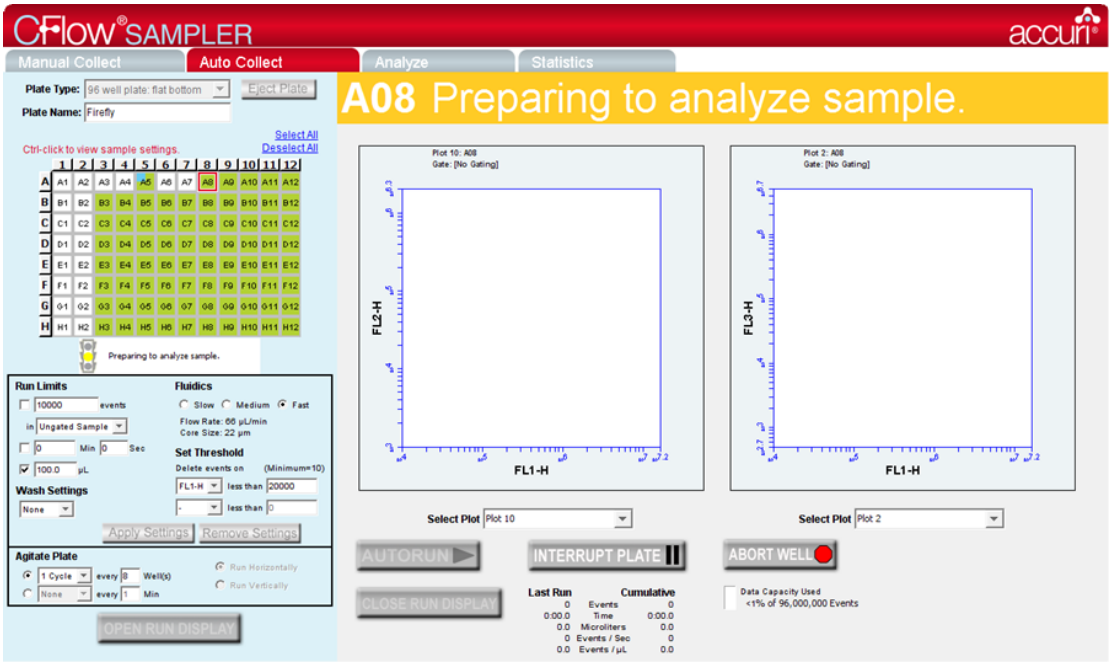
10. Click the "**Open Run Display**" button located underneath the Acquisition Control. Two plots will appear the right of the plate layout.



11. Again, ensure that the Acquisition Controls display the following settings before proceeding.

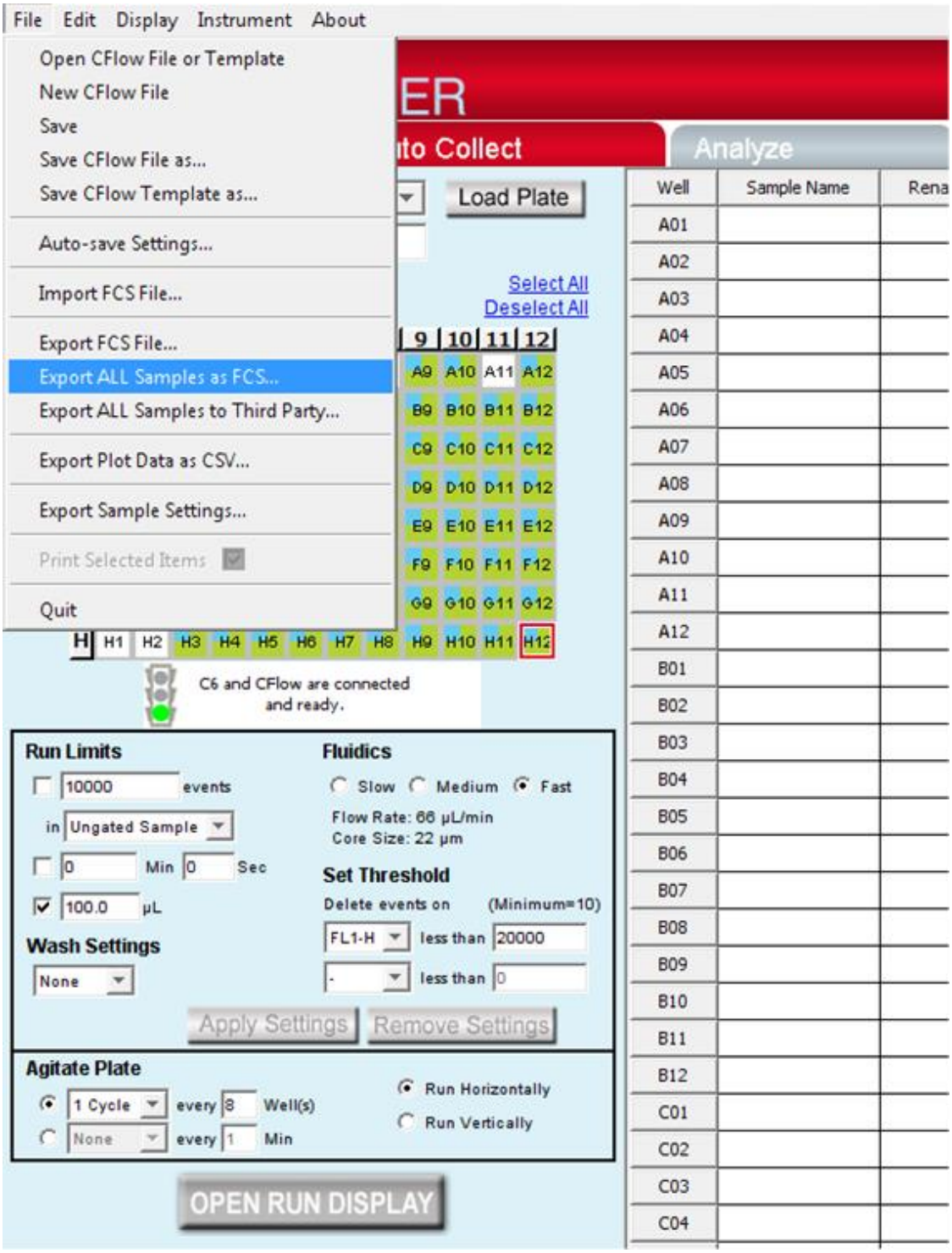


12. Click the "**Auto Run**" button located beneath the first plot. The cytometer will now begin automatic acquisition of the first selected well. Once the well has been acquired, it will be marked with a small blue square and the sampler will move on to the next well.



13. After all samples have been acquired, click the "**Close Run Display**" button.

14. 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. Load the exported FCS file into the Firefly™ Analysis Workbench for analysis.


File Edit Display Instrument About
Open CFlow File or Template
New CFlow File
Save
Save CFlow File as...
Save CFlow Template as...
Auto-save Settings...
Import FCS File...
Export FCS File...
Export ALL Samples as FCS...
Export ALL Samples to Third Party...
Export Plot Data as CSV...
Export Sample Settings...
Print Selected Items
Quit
ER
to Collect
Analyze
Load Plate
Select All
Deselect All
C6 and CFlow are connected and ready.
Run Limits
10000 events
in Ungated Sample
0 Min 0 Sec
100.0 µL
Wash Settings
None
Fluidics
Slow Medium Fast
Flow Rate: 66 µL/min
Core Size: 22 µm
Set Threshold
Delete events on (Minimum=10)
FL1-H less than 20000
less than 0
Apply Settings
Remove Settings
Agitate Plate
1 Cycle every 8 Well(s)
None every 1 Min
Run Horizontally
Run Vertically
OPEN RUN DISPLAY
Well
Sample Name
Rena