Beckman Coulter Cytoflex Start-up, Shut down, and QC SOP

Equipment: Beckman Coulter Cytoflex
S. No: AW24148
Location: TRB Fourth Floor A4-143
Description: 3 lasers (405nm, 488nm, 640nm), has the flexibility to allow 10 colour experiments and simple reconfiguration with filter swapping. It uses a 96 well HTS plate loader module accepts U, V or flat bottom plates.Uses analysis software (CytExpert)

Location: TRB Fourth Floor A4-143A

CytoFLEX Configuration

Cytoflex (B-R-V)			
Laser	Bandpass	Parameter	
Blue (488nm), 50mW	488/10	"SSC"	SSC
	525/40	"FITC"	FITC, BB515, AF488
	585/42	"PE"	PE, BYG584
	610/20	"ECD"	PE-CF594, PE-Dazzle594, PE-Texas Red, ECD
	690/50	"PC5.5"	BB700, PerCP, PerCP-Cy5.5, PerCP-eF710
	780/60	"PC7"	PE-Cy7
Red (638nm), 50mW	660/20	"APC"	APC, AF647
	712/25	"APC-A700"	AF700, APC-AF700, APC-R700
	780/60	"APC-A750"	eF780, APC-Cy7, APC-eF780, APC-AF750, APC-H7
Violet (405nm), 80mW	450/45	"PB450"	BV421, eF450, V450, PacBlue, SB436
	525/40	"KO525"	BV510, BV480, V500
	610/20	"BV605"	BV605, SB600
	712/25	"BV711"	BV711, SB702
	780/60	"BV786"	BV786, BV785, SB780

CytoFLEX Daily Start-Up:

-If necessary, fill the sheath fluid container with CytoFLEX Sheath Fluid not exceeding the maximum volume indicated (4 L). The boxes containing Milli-Q water are located next to the unit.

-If necessary, empty the waste container and add two bleach tablets to the empty container. The tablets are also located next to the unit.

-Turn on the main power switch located on the back left cover of the Cytometer.

-Log into the BCCHR network and the Windows operating system and select the CytExpert desktop icon to open the software. 

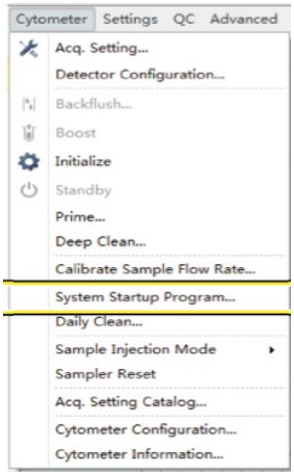
-Log into the software using your lab ID (ask your teammate/Lisa if you need your lab login information)

-Verify that the connection indicator light in the lower-left corner of the software screen is green, and Connected is displayed.

Verify that the Sheath and Waste flow indicators in the lower right corner of the software screen are green, indicating that the fluidics system is standard. If the light turns red, the sheath is detected as empty or waste as full

System Startup Program

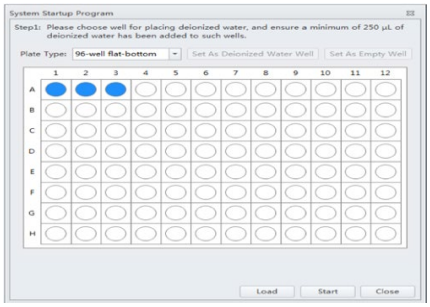
Select System Startup Program in the Cytometer menu.



-The plate loader automatically ejects the plate holder stage, and the System Startup Program window appears.

To load a regular 96w U-bottom plate, add to white plate holder, ensure the plate is flush with holder, and ensure plate holder is flush with the stage (jiggle the plate and white plate holder to ensure it doesn't move significantly). Otherwise, the probe can read the wrong well / hit a wall in the plate, / be damaged (some of these have happened before)

-Follow the software prompts and select three wells as Deionized Water to perform an initial wash.



-Select "Load" to load the plate.

-Select "Start" to start the program.

-Confirm that the correct plate is placed correctly by pressing OK. This step takes about 8 minutes

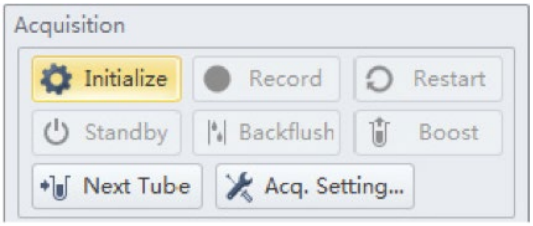
-When the system finishes acquiring the selected well, it uses the remaining time to warm up.

-When warm-up is complete, the plate loader ejects the plate holder stage.

-Select Close to quit the start-up program. The system is now ready.

Initializing the Instrument

-Select Initialize in the Data Acquisition Control screen or Initialize in the Cytometer Menu to initialize the instrument.



NOTE After the instrument initializes, the laser powers on, and the fluidics system begins to function. The laser powers on to achieve operating status in the initialized state, and the sheath fluid flows.

1. If you need to execute a task with the Fluid Containers (like adding more buffer or dumping the waste), do so with the instrument in the standby state.
2. If the instrument remains idle for 10 minutes, the Cytometer automatically enters the standby state.

CytoFLEX Quality Control:

Workflow:

Prepare the QC sample	→	Enter the QC experiment	→	Select target values	→	Acquire data	→	Confirm results

-Take one 96-well plate and label one well as the QC sample.

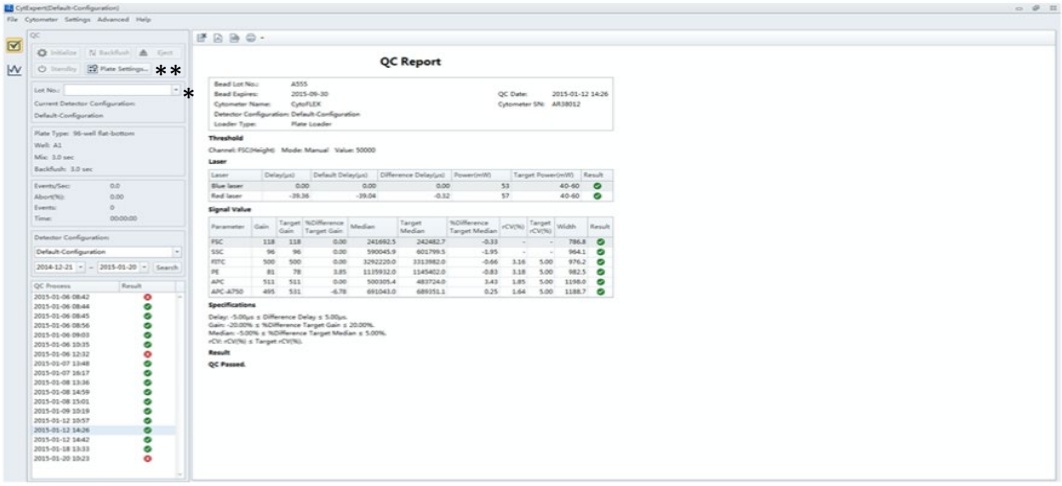
-Vortex or shake thoroughly mix the bottle of CytoFLEX Daily QC Fluorospheres (Cat# B53230). This vial is usually located in the flow core fridge by the door.

-Add one drop of QC Fluorospheres to one well of the plate.

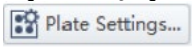
-Add approximately 200 µL of deionized water to the sample well. Place the plate in a dark location at 2-8 °C until ready to load into the instrument for QC. Keep the plate in the same fridge.

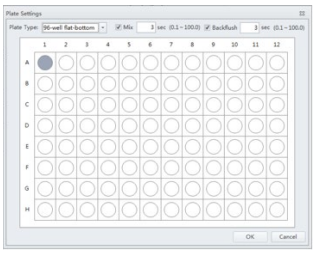
-Open the CytExpert QC screen

-Select Start QC in the QC menu to access the QC experiment.



*Ensure that the QC bead lot number is selectable. Dropdown menu.

**Insert the prepared QC plate into the plate holder and select the "Plate Settings" icon, and a window will appear. 



-Select the appropriate well containing QC beads

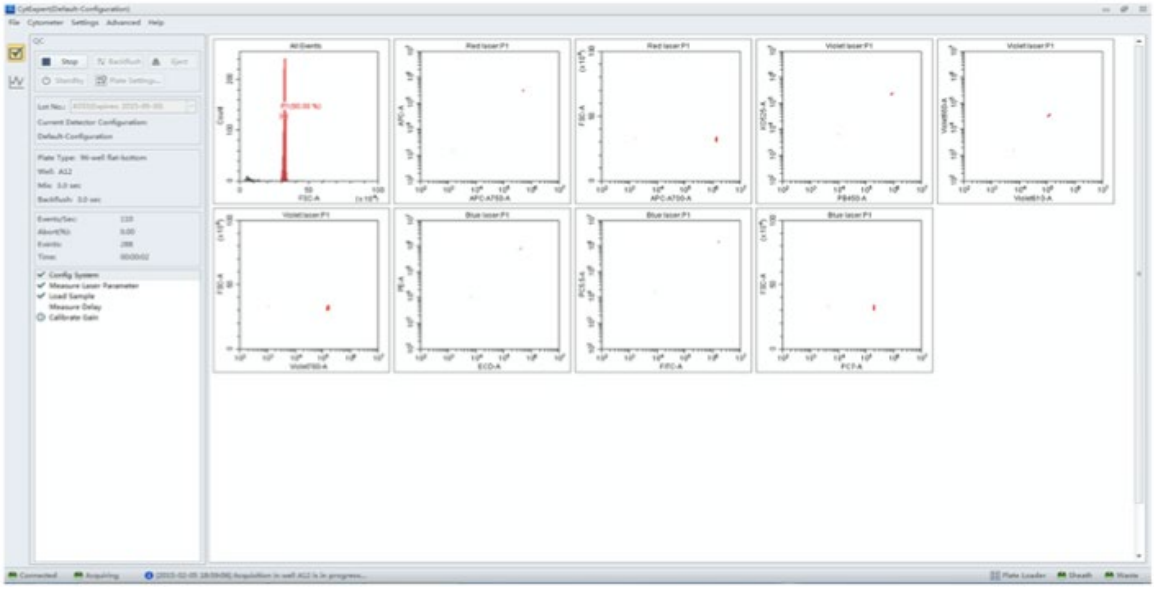
-Select the desired plate type from the drop-down menu.

-Select "Load," and the plate goes into the instrument.

-Select "Start" to load the sample and begin to run the QC.

-Confirm that the correct plate is placed correctly and hit OK.

-Completed processes appear on the left. Plots appear on the right. The QC experiment sequentially detects configuration, laser power, laser delay, signal strength, and coefficient of variation.



-Select a QC run from the QC Process list on the left, and a QC report appears on the right.

QC Process	Result
2015-10-27 11:08	✗
2015-10-27 11:11	✓
2015-10-28 11:45	✓

-Store the plate in the fridge as this dilution of beads is suitable for 4-5 days on daily use.

NOTE The results column indicates a passing QC result with GREEN and a failed QC result with RED.

QC results must meet the following criteria to pass:

- The gain differences must be ≤20% from the target gain.
- The median fluorescence intensity (MFI) differences must be ≤5% from the target MFI.
- The rCV must be ≤5%.

  NOTE The CytoFLEX Daily QC Fluorospheres rCV pass QC criteria is ≤5%.

CytoFLEX Troubleshooting:

QC Troubleshooting:

- Double-check that lot # for the beads are the same indicated on the QC window

- Make sure beads are not expired nor kept out too long in the light or at room temp

  (diluted beads can be kept in the fridge in the dark for up to 5 days)

- Prime twice and Re-run QC

- If you get the error message that the event rate was too low – add more beads

- If you get the message that it wants to change the laser delay, don't accept changes

- If the problem persists, run a daily cleaning and re-run the QC

- If, after all of this, QC still doesn't pass, call for service.

Generic Fluidics Troubleshooting:

- Backflush a few times (rinses the probe and sample line)

- Prime (need to be in Standby) (flushes the flow cell with sheath)

- Daily Clean (use Flow-Clean or 10-50% Contrad as cleaning agent)

- Deep Clean (need to be in Standby), let sit for at least 30min, then Prime

- If you are suspicious that the probe is clogged, remove the probe and replace it with a new one (or sonicate the probe in a tube of water for 2 min)

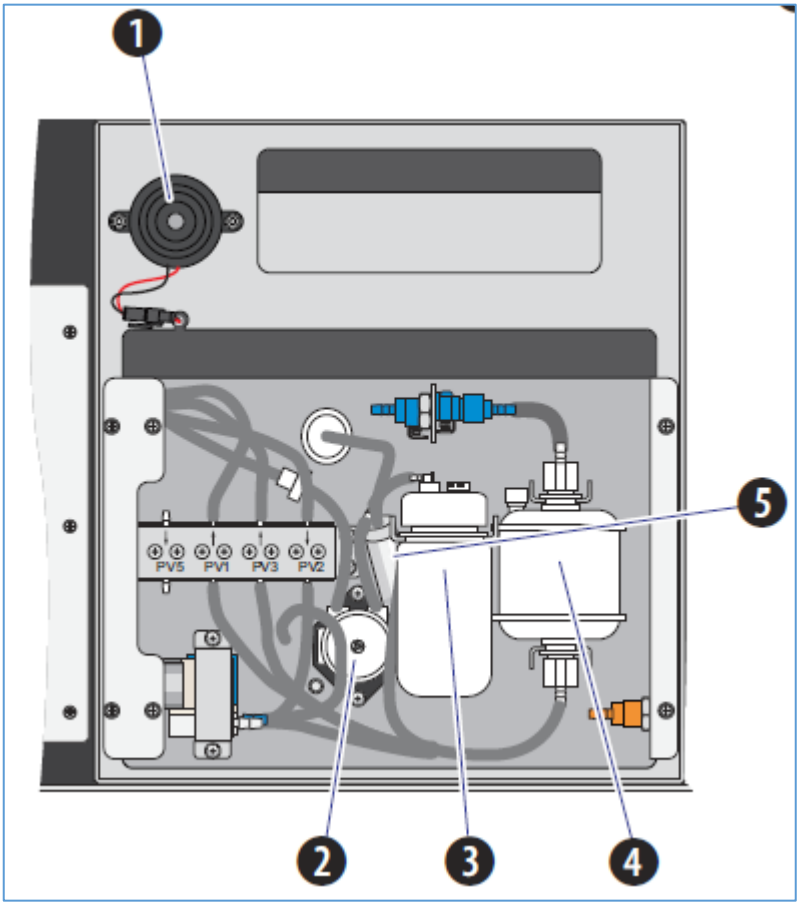
- Check the sheath damper – should not be more than 1/3 – ½ full

- Make sure sheath & waste connections are harness and not kinked

- Change peristaltic pump tubing (and then recalibrate sample flow rate, especially if doing volumetric absolute counts)

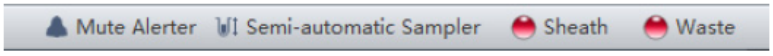
CytoFLEX Maintenance

- ***Every Day:***
    - Daily Clean (using Flow-Clean and water): After QC, in between experiments, and at the end of the day
- ***Once a month:***
    - Deep clean = cleans flow cell = uses 50% Contrad 70 solution
        - Shouldn't leave Deep Clean solution in the flow cell for more than 24h
        - If the unit will be shut down and not used for more than ten days, it is recommended to complete one deep clean before resuming use.
    - Rinse the sheath container with new sheath, swish around to get all internal surfaces, discard, then fill with new sheath
    - Clean the waste container: fill with 1L diluted bleach, put the waste harness back in, let sit for 10min, rinse container, and probe with H2O
    - Wipe down surfaces with diluted bleach, followed by 70% ethanol
- ***Every six months:***
    - Replace peristaltic pump tubing (more often (e.g., Once every 1-2 months) if using machine repeatedly, and especially if doing volumetric absolute counts)
        - Daily Clean
        - Recalibrate sample flow rate (especially if doing volumetric absolute counts)
    - Replace sheath filter
    - Replace deep clean solution
        - Open the top lid, open the two thumbscrews for the right-side cover, lift the side cover up and out
        - Fill the bottle with 30 mL Contrad + 30mL DI water
    - Reset maintenance reminder
    - With the right-side cover removed, inspect for fluid leaks:
        - After initializing the instrument; After priming
        - Check sheath damper – shouldn't ever be full



1. **Alarm.** Emits a warning sound when there is a problem with the Fluid Container/Cubitainer capacity or with the performance of certain operations.

   NOTE When the alarm sounds, the Mute Alerter icon appears in the status bar. The alarm continues for about 30 seconds. To mute the alarm temporarily select the **Mute Alerter** in the status bar. The icon disappears when the waste container/cubitainer is emptied and/or the sheath container/ cubitainer is filled/replaced.



2. **Deep Clean solution peristaltic pump.** Transfers cleaning solution to the flow cell.
3. **Deep Clean solution bottle.** Contains the diluted cleaning solution that helps to clean the flow cell.
4. **Sheath fluid filter.** 0.2 µm filter, for filtering sheath fluid.
5. **Sheath damper.** Regulates the sheath flow rate and decreases the sheath flow fluctuation.