

FORTESSA Start, Shut down, and QC SOP

Equipment: BD Biosciences FORTESSA X-20 (5 lasers: B/R/V/YG/UV)
S. No: H656385C2001
Location: TRB Fourth Floor A4-143
Description: 18 fluorescence detectors with a total of 20 parameters with 405nm, 488nm, 561nm and 640nm lasers. Uses Diva 8 software. Upgraded with HTS plate loader in 2020

Reference:

<https://www.bdbiosciences.com/content/dam/bdb/marketing-documents/BD-LSRFortessa-X20-Cell-Analyzer-User-Guide.pdf>

Materials:

- CST Beads, BD Biosciences Catalog Number(#655051)**LOT number may vary**
- Sheath fluid
- Working dilution of FACS Cleaning Detergent, CAT#340345
- Distilled water
- FACS Flow Supply System

Consumables:

- BD 5 ml FACS tube, polystyrene

1.1 Before Start-up:

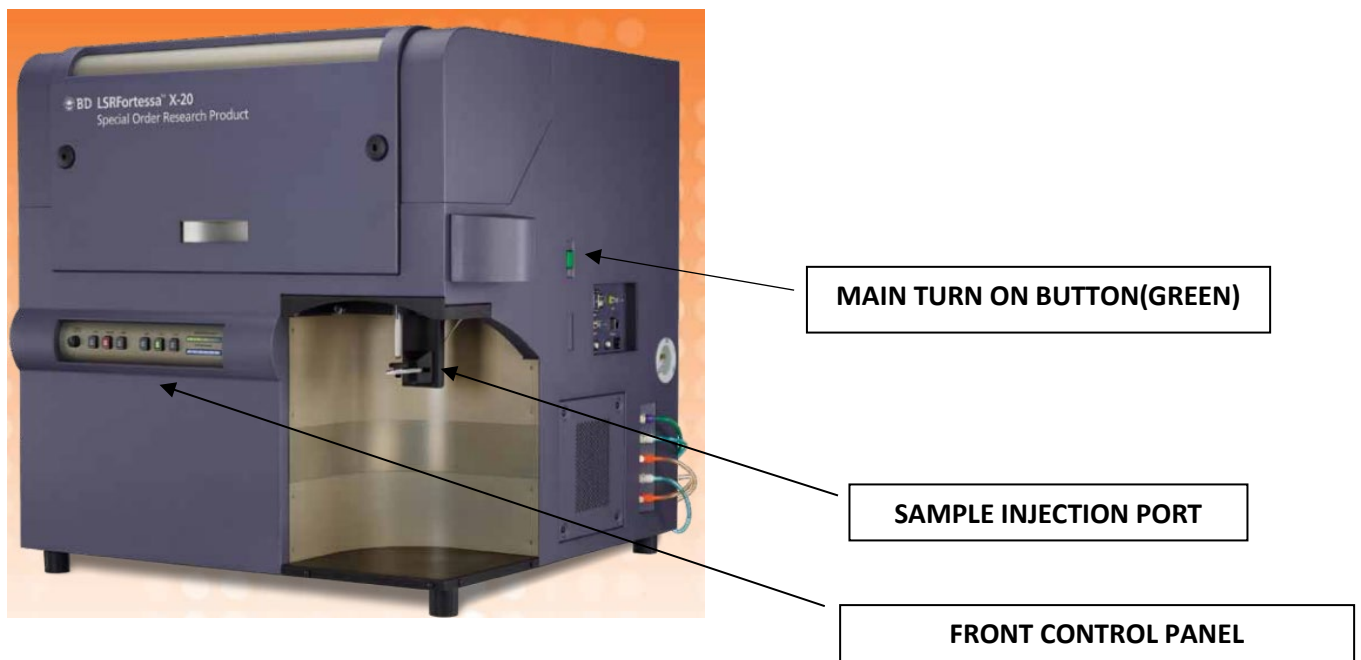
1. Check the waste tank located under the instrument. Make sure the waste tank is empty. To dump the waste tank, please follow the following instructions:

- If the waste tank is full, it is necessary to dump the fluid. **IMPORTANT:** before draining, disinfect the waste fluid.
- Change the waste tank and connect an empty waste tank.
- Add bleach tablet; 1 tablet per 7 Liter of waste fluid.
- Label the tank and add the date of bleaching.
- Leave the bleached tank overnight and drain the waste into the sink.

2. Check the level of sheath fluid located under the instrument. If needed, refill the tank with sheath fluid which is available beside FORTESSA in 10L White boxes.

Turn on the instrument, the green button on the right side of the device.

FIGURE 1: FORTESSA INSTRUMENT

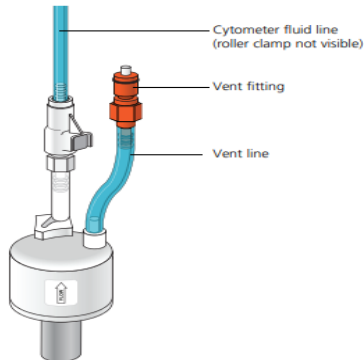


3. Check the filter behind the instrument to make sure there is no air bubble. If there is an air bubble, try to move the air bubble under the venting tube, press the orange valve, release the air, and leave the filter. Please see the following figures:

Removing trapped air bubbles:

To remove air bubbles:

1. Check the sheath filter for trapped air bubbles.

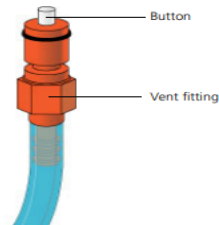


2. If bubbles are visible, gently tap the filter body with your fingers to dislodge the bubbles and force them to the top.



Caution! When removing air bubbles, do not vigorously shake, bend, or rattle the sheath filter or you might damage it.

3. Direct the vent line into a beaker and press the small button at the end of the vent fitting against the side of the beaker until a steady stream of fluid empties from the filter.



4. Tilt the filter and verify that no trapped air remains in the filter.
5. Repeat steps 3 and 4 until no air is observed in the filter.
6. Check the sheath line for air bubbles.
7. Open the roller clamp at the fluidics interconnect (if necessary) to bleed off any air in the line. Collect any excess fluid in a waste container.
8. Close the roller clamp.

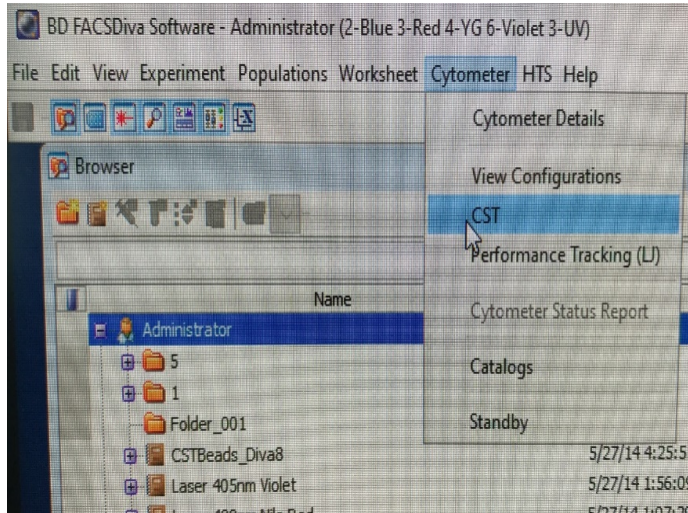
PRIME



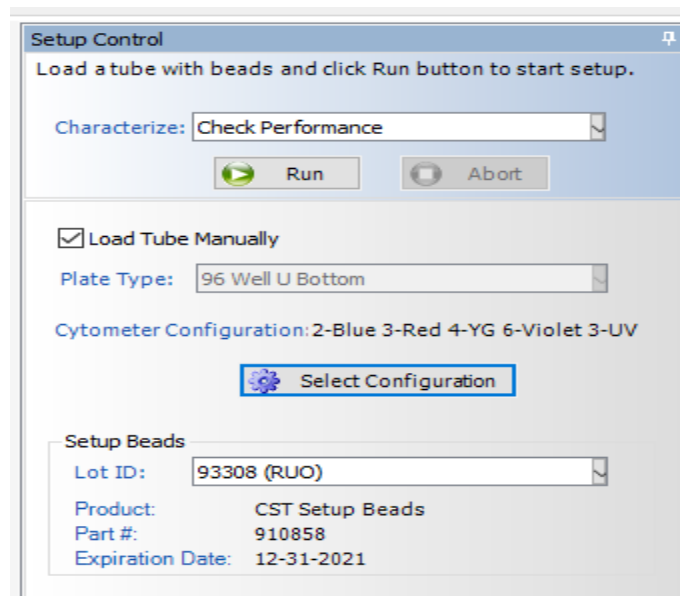
1.2 Cytometer startup and tracking:

4. Let the laser warm up before running the CST. It may take 10-20minutes.
5. Log in to the BCCHR using your username and password
6. CST beads vial designated for FORTESSA is kept in the fridge in Room A4-143A. Please communicate with the core manager Lisa Xu to know which lot is in use (all current lots for all cytometers are in a labelled plastic box inside the fridge).
7. Once you are informed about the lot# of CST beads, you need to make a working dilution. Take a FACS tube, add 1 ml of DW, add three drops of previously vortexed CST beads, then vortex again for a few seconds. Label the tube with the lot #, the date, and the instrument. Keep the diluted beads in the fridge protected from light.
8. Start cleaning the tubing by adding 3-4 ml of FACS cleaning solution into a FACS tube, insert it into the nozzle, set the fluid speed on high, and press the RUN button on the

- instrument's front control panel. 1 min with arm aside and 5 min with arm in the position.
- Start washing with DW: add 3-4 ml of DW in a FACS tube, insert it into the nozzle, set the fluid speed on HIGH, and press RUN button, 1 min with arm aside and 5 min with arm in the position.
 - Click on FACS Diva software, then log in to your laboratory username and password.
 - On the default FACS Diva open page, select cytometer > CST



- Wait until the RUN sign on the FACS Diva software turns green.



13. Make sure that the lot number ID matches with the beads you are using.
14. Then press the LOW button on the front control panel of the instrument and turn backward five times the fine tubing nob. Now, insert the CST beads tube into the nozzle, wait for 30 seconds, and click the green RUN on the FACS Diva.
15. The software automatically calculates the CV of the signals. It may take about five min. If everything goes well, a report shows up saying that QC passed. If it does not pass with warnings, it is necessary to start the troubleshooting process, section 5.2 p3.
16. Please remove the diluted CST beads tube, put it back in the fridge, and run cleaning as described in steps 11 & 12.
17. Press the LOW BUTTON, then press the STAND BY button on the control panel. Suppose there is no booking on the instrument, exit CST. Diva will go back to the default page, and a window comes up. Click on USE CST SETTINGS.
18. Click on FILE on the left corner, choose log out.
19. Log off from the computer and turn off the instrument; otherwise, leave it on for the following user.

1.3 TROUBLESHOOTING

ISSUE	ACTION
PASSED WITH WARNING	Ignore the warning IF the CV in the report is less than 7-8% on a few channels. However, it depends on your specific requirements for the experiment
IT DID NOT PASS; THE NUMBER OF BEADS IS TOO LOW	Make new diluted CST beads tube & rerun CST QC
DID NOT PASS	Run cleaning again and repeat CST QC
DID NOT PASS	Run the deep clean process and redo CST QC
DID NOT PASS	COMMUNICATE WITH LISA XU
Warnings on <2 channels %rCV <10	<ul style="list-style-type: none"> • Proceed with using a cytometer • Observe: is this a consistent trend (same parameter? Similar %rCV to previous checks?)
Warnings on >2 channels %rCV >10, <15	<ul style="list-style-type: none"> • Clean machine as described in point 1 above and check visually before proceeding • If the warning does not clear up, assess whether the affected parameter will affect the resolution
High CV on >2 channels %rCV >15	<ul style="list-style-type: none"> • Call BD Field Service hotline at 1(866)542-6663

