

BD LSR Fortessa Standard Operation Protocol **Machine Startup and Shutdown Procedures (For Experienced users only)**

Machine Start up:

- Refill the sheath tank and empty waste tank if necessary, make sure all the tubing are connected properly.
- Turn on the computer power and log in to window (User Name: **BDAdmin** Password: **BDIS#3\$\$**).
- Double click the **Tera Term**, select **Serial** and click **OK**.
- Turn on the Green Power button at the upper right of Fortessa.
- Remove air bubbles from the sheath filter.
- Load a tube of 3mL Rinsing solution (Solution 2)/MilliQ Water (Solution 3)/PBS, adjust the “Sample Fine ADJ” on the control panel to the maximum 10th round followed by running the solution at HI speed for about 30 minutes to startup the fluidics.
- Once the IP address appears in the Tera Term window, double click the **BD FACSDiva Software** → login to “**OCleaning**” account, no password required.
- The system is ready when it shows “Cytometer connected”.
- After 30 minutes of cleaning, adjust the “**Sample Fine ADJ**” anti-clockwise to the 5th round, change the flow rate to “LOW” and set the machine at “Standby” mode.
- Go to “**Cytometer**” → “**CST**”.
- Get the well-prepared CST beads from the fridge (prepare a new batch if needed: vortex the stock CST tube with black cap vigorously followed by adding 1 drop of the beads into 350ul PBS → mix well before loading). The CST tube must be labeled with **CST**, **lot number** and **date**.
- Once entering CST mode, BD FACSDiva software will be disconnected automatically.
- Double check with the CST lot number and check the box “Load tube manually”.
- Load the tube of CST beads and press “RUN”.
- Click “**Run**” and “**OK**” to initiate the CST test. When the CST test is completed successfully, click Finish and exit from the CST mode so that BD FACSDiva software can be connected. Unload the CST beads and load Solution 3. Return the CST beans to the fridge.
- Log out from the OCleaning account and log in to your own account for sample acquisition.
- If some parameters could not pass CST test, cleaning flow cell for longer period is needed.
 - a) Prime 2 times
 - b) Prepare one tube of 2ml FACS Rinse
 - c) Run 10 min at “HI” flow rate
 - d) Run CST again



Machine Shutdown:

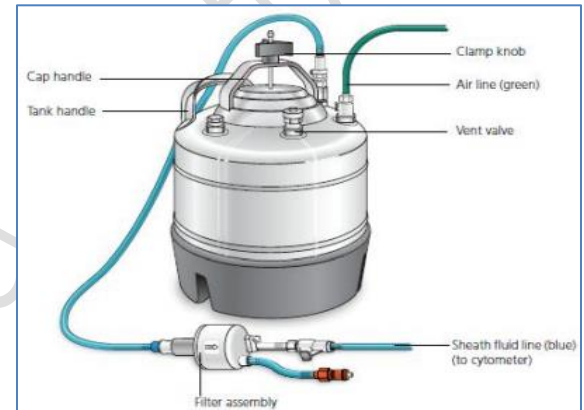
- After running through the whole process of machine cleaning, turn off the POWER button at the upper right of Fortessa.
- Exit the BD FACS Diva software and Tera Term → Shutdown computer.
- Refill sheath and discard the waste.

CPOS - Imaging and Flow Cytometry Core

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Refill Sheath Container

1. Make sure the flow cytometer is in STANDBY mode.
2. Disconnect the air line (green) and fluid line (Blue) from the sheath container.
3. Depressurize the sheath container by pulling up on the vent valve.
4. Remove the sheath container lid.
5. Unscrew the clamp knob and push down to loosen, if necessary.
6. Tilt the cap to the side to remove it from the tank.
7. Add sheath fluid (on the trolley) to the sheath container.
8. Fix the sheath lid and tighten the clamp knob to finger-tight.
9. Reconnect the air line (green) and fluid line (Blue).
10. Please pay attention to fluid line (Blue) to make sure it is not twisted or stretched.
11. Remove air bubbles from the sheath filter.



Empty Waste Tank

1. Make sure the flow cytometer is in STANDBY mode.
2. Disconnect the orange waste tubing and the black level sensor line from the waste container.
3. Take the waste tank to the sink.
4. Remove the lid of waste tank.
5. Empty the waste container.
6. Add approximately 0.5L of bleach to the waste in which it could cover the bottom layer of the container and tighten the lid.
7. Reconnect the orange waste tubing and level sensor line.

