Standard Operation Procedure for BD LSRFortessa X-20

1. **Check the sheath cube and the waste tank, and the FACS Flow Cart is on.**
    - If the sheath cube is low, remove the probe, change a new cube, replace the probe, and press "Restart" on the FACS Flow Cart.
    - If the waste tank is full, remove the probe and empty the waste. Add 300 ml of bleach, replace the probe and press "Restart" on the FACS Flow Cart.

2. **Turn on the computer. Log in to Windows as the BD operator. The password is "Welcome#1".** Double-click the DIVA icon to open the instrument acquisition software. Log in to the software.

3. **Turn on the instrument system power** by pressing the green button on the right side of the instrument.

4. Rinse the System (First user of the day)

a) Install a tube containing 3 mL of 100% Contrad70 solution on the SIP.

b) Press RUN and HI on the cytometer fluidics control panel.

c) Allow the solution to run on HI for 10 minutes.

d) After 10 minutes, change a tube containing 3 mL of BD Rinse buffer on the SIP.

e) Allow the solution to RUN on HI for 10 minutes.

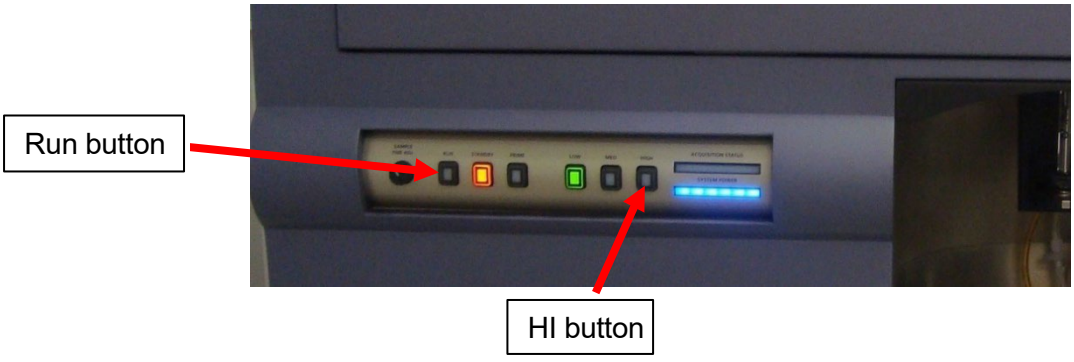
f) After 10 minutes, change a tube containing 3 mL of DI water on the SIP.

g) Allow the solution to RUN on HI for 10 minutes.

h) Press STANDBY button on the fluidics control panel.

i) Place a tube containing no more than 1 mL of DI water on the SIP.

j) Allow 30 minutes for the lasers to warm up before running samples or CS&T.

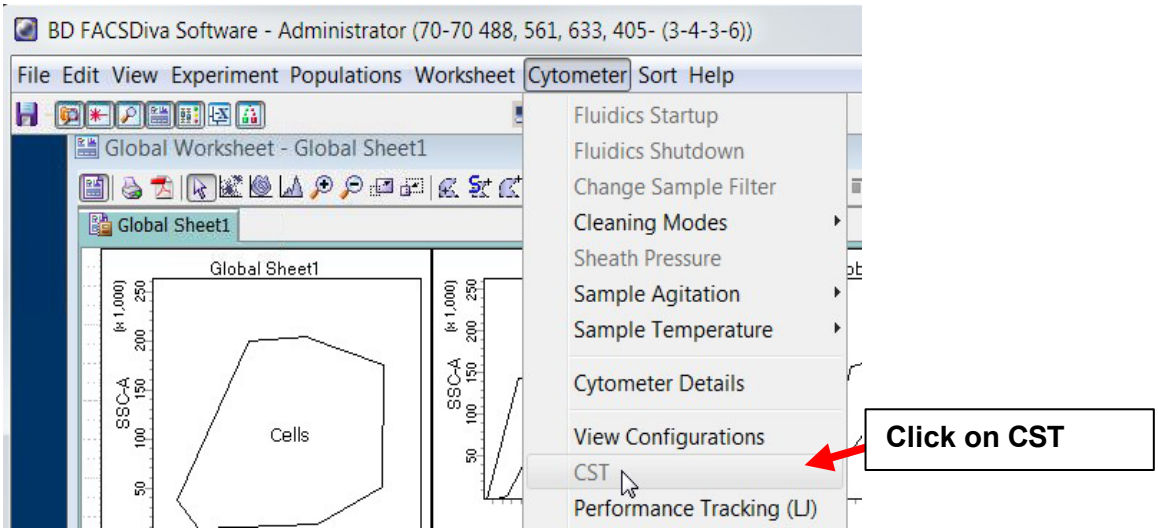


5. Running CST beads (First user of the day) (20 points)

a) Prepare CS&T research beads:

- 350uL filtered PBS + 1 drop of CST bead
- Vortex well

b) Select **Cytometer > CST**



c) **Verify that the lot ID under Setup Beads** matches the information on the BD FACSDiva CS&T research beads vial.

d) Install the pre-prepared CST bead tube on the cytometer

e) Select the **Load Tube Manually** checkbox

f) Remove the DI water tube from the SIP

g) Set the flow rate to LO and press RUN on the cytometer fluidics control panel

h) Click **Run** in the CST workspace, and click **OK** to confirm that the tube has been installed.

i) When the performance check is complete, remove the CST tube from the cytometer.

j) Install a tube with 1 mL DI water onto the cytometer and press STANDBY

k) In the **Cytometer Setup and Tracking performance** check completion dialog, click **View Report**.

l) Verify that the cytometer performance passed

m) Select **File > Exit** to close the Cytometer Setup and Tracking workspace and reconnect to the BD FACSDiva interface.

Important Notes:

- **QC is run by Core staff.**
- **You only need to run the QC only if Core Staff have not run it for the day.**
- **If you run the QC and if CST fails please contact Core staff via email.**

6. Shut Down

a) Install a tube containing 3 mL of 100% Contrad solution onto the cytometer, RUN HIGH for 5 minutes.
b) Install a tube containing 3 mL of BD FACSClean solution onto the cytometer with the support arm to the side (vacuum on).
c) Allow the solution to RUN on HI for 1 minute
d) Move the support arm under the tube (vacuum off) and continue to RUN on HI for 5 minutes.
e) Repeat b)-d) steps with full-strength BD FACSRinse solution
f) Repeat step b)-d) steps with DI water.
g) Install a tube with no more than 1 mL of DI water onto the cytometer
h) Set the fluidics mode to STANDBY.
i) Exit BD FACSDiva software.
j) Turn off the cytometer.
k) Log out the computer.

Important Notes:

- **DO NOT leave more than 1 mL of water on the SIP.** When the instrument is turned off or left in standby mode, a small amount of fluid will drip back into the sample tube. If there is too much fluid in the tube, it could overflow and affect the cytometer performance.
- **DO NOT turn off the power on the BD FACSFlow supply system (FFSS).**
- **Check the waste tank after use. Empty the waste tank if necessary.**