Flow Cytometry Protocol For BD LSRFortessa

(1). Check to make sure the sheath fluid tank has sufficient sheath fluid (the sheath fluid should be just below the top line). If not, fill up the tank to just below the top line.

(2). Check to waste bucket. If it is almost full, discharge the waste solution in the sink and add about 50 mL bleach into the bucket.

(3). Turn on the flow cytometry machine (push the square green button on the right side of the machine). On desktop, click open "BD Coherent Connection" and "BD FACSDiva Software". Both do not require password. Wait 25-30 mins for the machine and the lasers to warm up.

(4). Bleeding: Bleed the large glass jar underneath the machine. Then bleed the small glass jar on the right side of the machine.

(5). Prime: Pull the arm to the left side and remove the water tube. Change speed to "high", [then press PRIME button. After priming, the PRIME button will no longer be lit up, and the STANDBY button will lite up.] Repeat [...] for 2 more times. After the third priming, put the water tube back and close the arm.

(6). Setting Up CST:
i. Add 350 uL PBS into a glass flow tube, then shake well the BD Cytometer Setup and Tracking Beads bottle (4 degree fridge in the cell culture room). Then squeeze the bottle to add a single drop into the flow tube.
ii. Open the arm, take out the water tube, vortext the flow tube containing the beads for about 5-8 second, then load onto the machine, close the arm.
iii. Set the speed at "Slow", then on BD FACSDiva interface, click "Cytometry', then in the dropdown list, select "CST".
iv. In the new window "Cytometer Setup and Tracking", click "Run", then click "OK" for the dialog box.
v. On the machine, push "RUN", wait until the CST running is over, then press "STANDBY" on machine.
vi. On the BD FACSDiva interface, click "Finish", then on the new pop-up window, choose "Use CST Settings".
vii. Note: Often the CST running fails. In such cases, prepare new beads and repeat steps i-v.

(7). Preparing Cell Samples (12-well plate):
i. Remove the growth media from the well using P1000 pipet.
ii. Add 1000 uL 1XPBS, then remove the 1XPBS solution using P1000 pipet.
iii. Add 200 uL Trypsin, leave in the incubator for 3-5 mins, then add 800 uL complete growth media.
iv. Transfer the cell suspension to an Eppendorf tube, spin at 1000 rmp for 5 min, then use P1000 pipet to remove the supernatant.
v. Resuspend the cell pellet in 200 uL 1XPBS using P200 pipet. Transfer the cell suspension to the flow tube. Add another 300 uL 1XPBS to the flow, then leave the tube on ice.

(8). Running A New Experiment:
i. Open an exisiting file, right click and select "Copy without existing data".
ii. Use a positive sample to adjust the laser voltage setting.
iii. To run a sample, choose the right tube name on the Specimen list, double-click to open that sample.
iv. Voretx the sample flow tube, open the arm, load the sample, close the arm. Then click "RUN" on the machine, then click "Acquire" on the FACSDiva interface. If the SSC v. FSC appears normal, click "Record" on the FACSDiva interface. Collect 50,000-100,000 event.
v. Once sufficient events are collected, Click "Record", "Acquire" on the FACSDiva to stop the program. Then click "STANDBY" on the machine.
v. Open the arm. Remove the flow tube, then repeat steps iii-v for all other samples.

(9). Cleaning:
i. After the experiment, load onto the machine the flow tube containing bleach, close the arm, change the speed to "High" on the machine, click "RUN" on the machine, run for 4 mins. Then open the arm, click "RUN" on the machine, run for 1 min.
ii. Unload the bleach tube, then load onto the machine the flow tube containing detergent, close the arm, click

"RUN" on the machine, run for 4 mins. Then open the arm, click "RUN" on the machine, run for 1 min.
iii. Unload the detergent tube, then load onto the machine the flow tube containing water, close the arm, click "RUN" on the machine, run for 4 mins. Then open the arm, click "RUN" on the machine, run for 1 min.

(10). Turning Off The System:
Close the "BD FACSDiva Software" and the "BD Coherent Connection". Turn off the machine. Make sure to record in the log book for flow cytometry.