SWITCHING ON THE HTS SYSTEM

1. **Turn ON the system** (see in this folder *FORM.UCF.043 - BD LSRFortessa Operation*)
2. **Check Fluidics Levels:** PBS and H_2O – Fill them if needed.
3. Remove the tube with Water.
4. Switch the acquisition control to plate mode.
5. Move aspirator arm to the side and unscrew sleeve (clockwise) and remove it.
6. Attach the SIT protector.
7. Attach the sample coupler to sample injection probe (SIP). Push the sample coupler in place and tighten the top nut.
8. Turn on the HTS and press RUN.
9. Launch BD FACSDiva software using your own password and click “Use CST Settings”.
10. In Diva select **HTS > Re-initialize**. During the initialization, check for leaks on each of the 2 syringe pumps and also at the SIT where the HTS is connect via the sample coupler. **If needed, prime HTS unit (HTS > Prime) until there are no bubbles in the system.**
11. Define loader settings and acquire your plate.

GUIDE TO DEFINE LOADER SETTINGS

Generate experimental setup in the plate view window and define “Throughput mode”
1) **Standard** - slow but you can precisely define sample volume to be processed
2) **High** - very fast because. In this mode the HTS always aspirates 22 µl/well independent of the sample injection volume you choose. You can define to record 2-10 µl out of this.

Loader Settings:

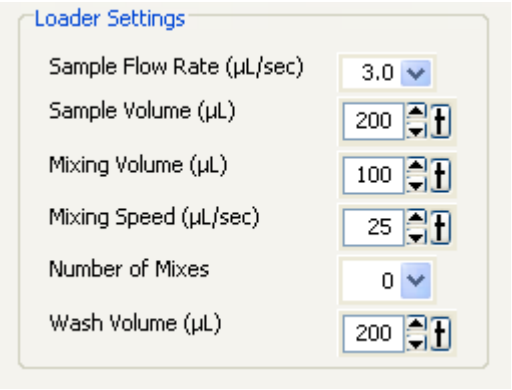
- **Sample Flow Rate** (µl/s): is the speed the syringe injects sample on the cytometer in µL/second. You should not have more than 10.000 evts/s.
- **Sample Volume** is the volume of sample aspirated from each well for acquisition. During acquisition in standard mode, HTS aspirates the selected sample volume **plus an additional 20 µL from the well.**
  Make sure each well contains sufficient sample for the entered volume plus the dead volume. Insufficient volume can introduce air bubbles into the system.

BD recommends that you prepare your plate with a **minimum of 250 µL of sample/well for a 96-well** plate in standard mode, 100 µL/well for a 96-well plate in high throughput mode, and 50 µL/well for a 384-well plate (either mode).

- **Mixing Volume** is the volume of sample aspirated and dispensed during mixing. Make sure each well on your plate contains sufficient sample for mixing. BD recommends a **mixing volume that is one-half the available volume**.
- **Mixing Speed** is the speed that the syringe aspirates sample and dispenses sample to the well during mixing. This parameter is user specified depending on cell type and experiment layout.
- **Number of Mixes** is the number of mixing cycles that are performed before a sample is aspirated. This parameter is user specified depending on cell type and experiment layout.
- **Wash Volume** is the volume of sheath fluid dispensed for rinsing between wells. This parameter is user specified depending on cell type and experiment layout, **if using PI please use maximum wash volume (800 µl)**

SHUTTING DOWN

1. Run the cleaning procedure:
    a) Prepare a cleaning plate with:
        3 wells with **300µl** FACSClean
        3 wells with **300µl** H_2O Azide
        3 wells with **300µl** FACSClean
        3 wells with **300µl** H_2O Azide
    b) Run the selected wells with the following settings:



2. Prime HTS unit (HTS menu à Prime) 1X
3. Place cytometer in Standby mode
4. Switch off the HTS power
5. Detach the sample coupler from the cytometer SIT
6. Remove SIT protector

7. Reinstall the DCM sleeve
8. Install a tube of Water on the SIT and place the tube support arm under tube.
9. **Switch to acquisition tube mode.**
10. **Refill de PBS container**
11. If during your acquisition, **the waste container gets full, a HIGH PITCH SOUND will go off**. You should disconnect the black sensor (the noise will stop). As soon as possible, when convenient, put the cytometer in **Standby**, and **disconnect the orange connector**. Close the container with the red cap that is on top of it and replace the container with a new one.

12. **Turn OFF the system** (see in this folder *FORM.UCF.043 - BD LSRFortessa Operation*)