Switching on

1. **Check Fluidics Levels:** refill the PBS container and empty the waste tank.
2. Remove the tube with FACSRinse and press Prime 5x.
3. Switch the acquisition control to plate mode.
4. Move aspirator arm to the side and unscrew sleeve (clockwise) and remove it.
5. Attach the SIT protector.
6. Attach the sample coupler to sample injection probe (SIP). Push the sample coupler in place and tighten the top nut.
7. Turn on the HTS and press RUN.
8. Launch BD FACSDiva software using your own password and click "Use CST Settings". Note: if you switch on the HTS with the software running you must reinitialize the HTS (HTS menu→ reinitialize).
9. Prime HTS unit (HTS menu → Prime) 3X (make sure there are no bubbles in the system).
10. Prime the cytometer 3X, define loader settings and acquire your plate.

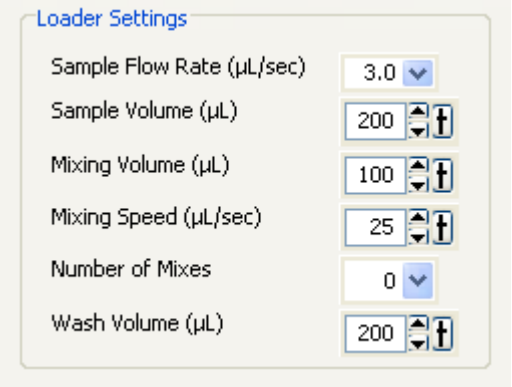
Running on HTS mode; guide to define loader settings:

- **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** FACSRinse
        3 wells with **300µl** H_2O Azide
    b) Run the selected wells with the following settings:



2. Prime HTS unit (HTS menu → 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 FACSRinse on the SIT and place the tube support arm under tube.
9. **Switch to acquisition tube mode.**
10. **Check Fluidics Levels:** refill de PBS container and empty the waste tank.