

INSTITUTO DE MEDICINA MOLECULAR JOÃO LOBO ANTUNES
FLOW CYTOMETRY FACILITY
HTS OPERATION

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Switching on the HTS system

1. Turn ON the Cytometer.
2. Check Fluidics Levels: PBS and H₂O – Fill them if needed.
3. Remove the tube with Water.
4. Switch the acquisition control to plate mode (on the right side of the cytometer).
5. Move aspirator arm to the side, 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 button (on the back of the HTS) and press RUN.

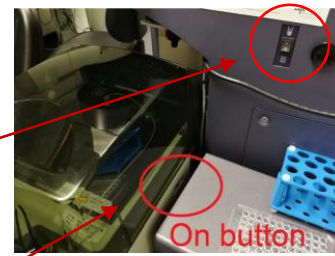
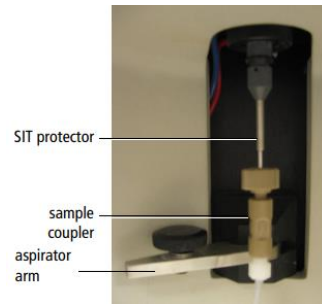
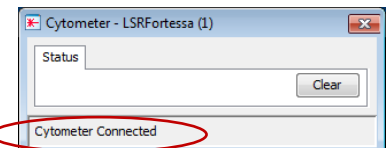


Plate / Tube mode button



Computer and Software Login

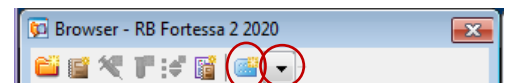
- Login on windows: Username: Flow Cytometry User
Password: BDIS
- Start the FACSDiva software: double-click the shortcut icon on the desktop.
- Login on DIVA using your own login name and password.
- Wait for the cytometer connection and click “Use CST Settings”.



NOTICE: To verify the workstation has successfully connected to the cytometer, check that the Cytometer window displays the message “Cytometer Connected” or “The system is ready” at the bottom of the window. If the message reads “Cytometer Disconnected,” switch the cytometer power off, wait 10 seconds, and then switch the power on. Restart the computer.

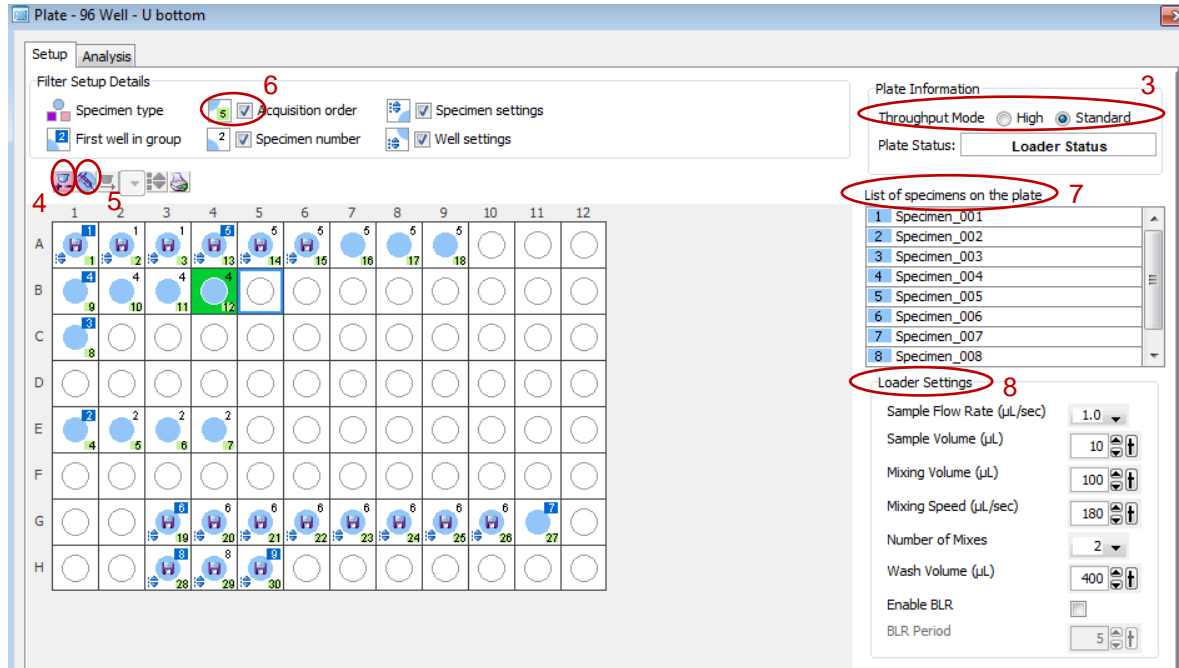
Setting Up Your Experiment

1. Create a new experiment, a specimen and open a tube.
2. Select the parameters you will use in your experiment and define your graphics and gates on the worksheet.
3. On 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.
4. Create a plate (1) and choose the correct type (2) (96/384, flat/round/V bottom) via the dropdown menu next to the “New Plate” button in the browser.
5. Generate an experimental setup in the plate view window and define “Throughput mode” (3):
 A) Standard: slow but you can precisely define sample volume to be processed (~20 µl dead volume + defined sample volume);



1 2

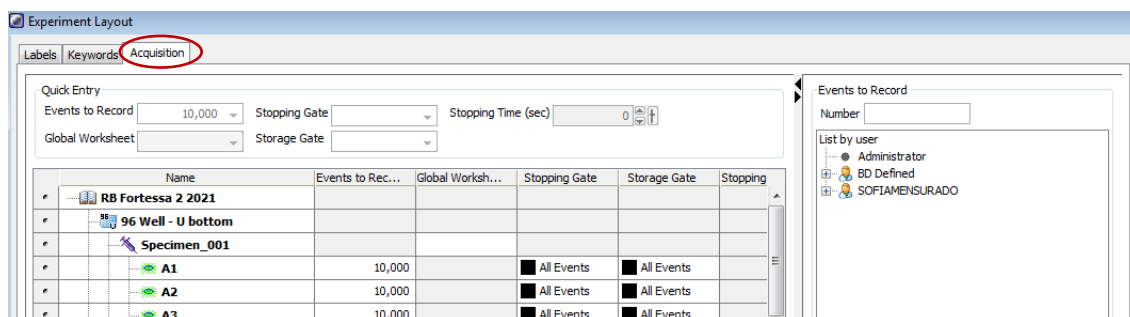
B) High: fast (one plate is read in 15 minutes) since it runs two pumps in parallel (sample injection/washing). In this mode, the HTS aspirates 22 μl /well independently of the sample injection volume you choose.



6. If you are going to perform settings and create compensations, select a well or group of wells necessary for that purpose, and then click the “Add Setup Controls” button (4) to add well(s) to the plate layout for adjusting cytometer settings. The wells are for setup only, and will not be recorded. Proceed adjusting the voltages and calculating the compensations as you would in tube mode. If preferred, the settings and compensations can be performed in tube mode and only after, switched to plate mode for acquisition of samples.
7. To create tubes for acquisition, select a well or group of wells, and then click the “Add Specimen Wells” button (5) to add a specimen to the plate layout. Wells will run in the order they are selected (6). You can also select the all plate if you want.
8. To rename specimens (7): click once to select the specimen name in the list of specimens on the plate, enter the new name for the specimen, and then press Enter.
9. Finally, determine the loader settings (8) appropriate to your experiment (only if you are using the standard mode).

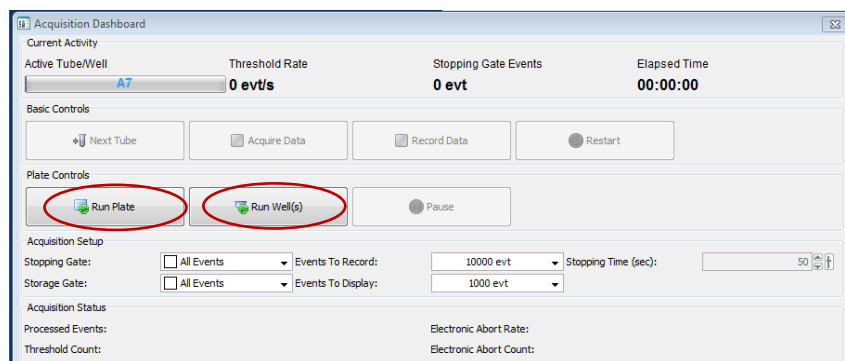
Loader Settings:

- 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).
10. To define the number of cells that you wish to acquire per well, open the “Experiment layout” from the “Experiment” menu and add the number of cells to be acquired in each well. Do not exceed 1M cells per well! This will result in a plate memory error.







Acquisition of samples

1. Remove the cover on the HTS and place your plate in the correct position. Be careful when closing the cover to avoid treading the white tube.
2. On the acquisition dashboard, select “run wells” if you only want to acquire a selected number of wells or “run plate” if you want to acquire the all plate. The cytometer will not only acquire but record it as well. Note: In plate mode, it is not possible to append the data acquisition. To reduce contamination from one well to another, increase the wash volume and adjust your sample concentration. If that is not enough, place wash wells between the sample wells. If populations look broader than expected, reduce the sample flow rate. Be aware that you should not exceed 1M cells per a 250uL well.



3. When the plate is finished, you can either switch for another plate or start the cleaning procedure if you finished your acquisitions for the day.

HTS acquisition symbols:

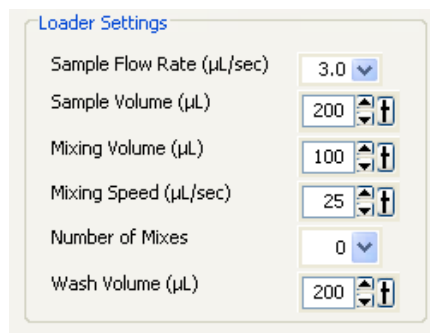
-  Acquiring.
-  Recorded.
-  Selected for acquisition and contains previously recorded data from aborted run.
-  Selected for acquisition, aspirated, and aborted.

Shutting down

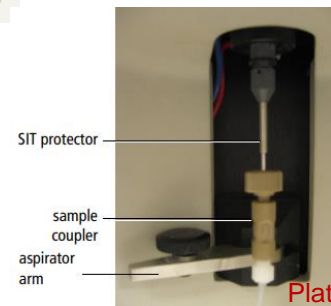
1. Run the cleaning procedure:

- a) Prepare a cleaning plate with:
 - 3 wells with 300µl FACSClean
 - 3 wells with 300µl H₂O Azide
 - 3 wells with 300µl FACSClean
 - 3 wells with 300µl H₂O 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 (on the back of the HTS)
- 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 (on the right side of the cytometer).
- 9. Refill de PBS container
- 10. 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.



- 11. Turn OFF the system if you are the last user of the day or if there is a gap of 3h or more between you and the next user, Perform the shut down as described on the right lateral of the cytometer.