General note

Using the Fortessa HTS requires a separate introduction. Please contact the facility at info@cytometry.uzh.ch to schedule an appointment.
Do not use the HTS if you need to analyze every cell in your sample because the HTS system will discard some sample due to dead volume in wells and tubings.
Inject sample volume is discarded once the Fortessa reaches the stopping criteria (e.g. # events to record). For this reason we recommend to record compensation samples and controls in tube mode.

Sample Preparation

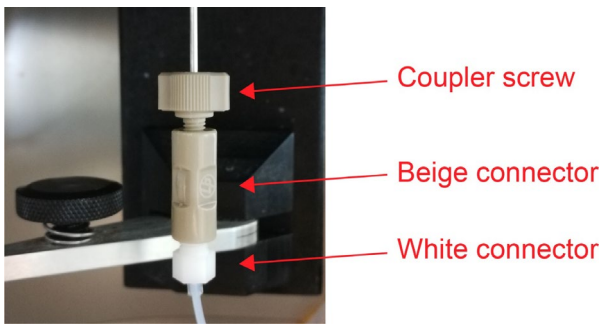
- For initial tests, use a large enough sample volume (≥100 µl/well). To calculate the total sample volume needed, you need to take the dead volume and the excess aspiration volume into account. The dead volume in the well is ~35 µl (varies slightly depending on plate type). This is the volume the needle cannot reach. When sample is aspirated, the HTS will aspirate more than the specified volume:
  - In Standard mode: 20 ul
  - In High mode: fixed aspiration volume of 22 ul
- Filter your cells prior to analysis to avoid sample line clogging.
- To ensure efficient runs optimize your cell density to achieve ~2'000-10'000 events/s ($10x10^6$ cells/ml is a good starting point for PBMCs; start optimizing for cell lines from $1x10^6$ cells/ml).

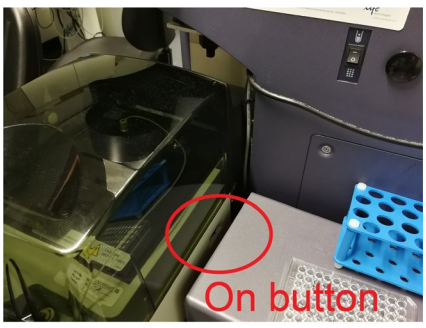
Starting the Fortessa using the HTS

- Switch on the Fortessa and wait until "Cytometer connected" appears in Diva.
- Prime the Fortessa 2× using the button on the instrument.
- Check the FACS Flow supply and the waste level of the Fortessa. You want the sheath tank at least ¼ full and half an empty waste so you do not have to interrupt your plate run.
- Switch Fluidics on by pressing the run button on the instrument.

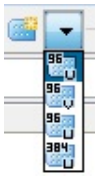
- Switch button to plate mode.
- Attach the "HTS Sampler Coupler" to the bottom of the sample needle. First, ensure that the white connector is securely screwed in to the beige connector. Next, screw the beige coupler screw into the beige connector. Then, unscrew the beige coupler screw ½ turn so that it is loosened. In this loosened state, slide the sample coupler onto the SIT until it reaches a soft stop – THEN push slightly more until it reaches a hard stop (hold on the sample needle to support stability). Finally, hold the lower part of the beige section with one hand while tightening the beige coupler screw with the other hand.

  If the coupler is salty dismantle it and rinse the thread with water.





- Make sure the HTS device is on by checking the power button on the back (see above).



- 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.
- Define basic settings for the experiment (create experiment, select appropriate channels and parameters).
- Create a plate of the correct type (96/384, flat/round/V bottom) via the dropdown menu next to the "New Plate" button in the browser.
- 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 (~30 µl dead volume + defined sample volume)
  2) *High* → fast because it run 2 pumps in parallel (sample injection/washing). In this mode the HTS aspirates 22 µl/well independent of the sample injection volume you choose. You can define to record 2-10 µl out of this.
- Create Specimen e.g. set up controls, compensation samples and normal samples. Recommendation: use of 2-3 "setup wells" for setting up FCS/SSC and other PMTVs or even better adjust scatter and fluorescence PMTVs beforehand in the tube mode.

  Note: Compensation wells need to start with A1 position. Otherwise compensation wells will not be recognized by the Diva software.
- Start measurement by "Run plate" or by selecting specific wells and "Run wells". If you cannot see plate control buttons in the "Acquisition Dashboard right click on the "Acquisition Dashboard" and activate "Plate Control".
- Prepare the cleaning plate with 250 µl FACSClean in A1-A4, 250 µl FACSRinse in B1-B4.
- In Diva select "HTS" → "Clean" and follow instruction (the cleaning requires about 10 min).
- Perform "HTS" → "Prime" to rinse the HTS with H_2O.

Switching back to Manual Loader for the next user

- Switch the Fortessa to Standby mode.
- Switch back to tube mode and shut off the HTS power via the button in the back.
- Unscrew the HTS sample coupler from the sample needle and TIGHTEN the screw again so the system doesn't leak water.
- Place a dH_2O water tube on the SIT and bring the sample support arm back into place.
- Clean HTS cleaning plate with dH_2O and store next to HTS.

Trouble shooting the HTS and optimization

- Clogs are most likely to happen in the sample needle of the Fortessa (not within the HTS!).
- Check coupler for liquid build up → disconnect, dry coupler, re-attach until resistance point, tighten.
- Prime HTS 2× to flush out any potential air in the tubing.
- Check that H_2O line of HTS is not jammed in under the cover of the HTS.
- Vortex plate or increase resuspension cycles before injection.
- Optimize sample concentration (remember that the minimum speed of the HTS 0.5 µl /sec equals a MEDIUM flow rate setting in tube mode) → HTS is very sensitive to blockages → if you have "sticky" samples use some Clean & dH_2O wells in between your samples and make sure you clean well in the end!
- Check that you have enough volume in the wells, air bubbles will render the whole system

useless → prime if necessary.

- Note: in plate mode it is not possible to append the data acquisition.
- The recommended mixing volume is half the well volume. High mixing speeds can lead to increased carry over.
- To test for carry over, prepare a well with just buffer after a sample well. The cells that you detect for that well are carry over from the well before.
- To reduce carry over, 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. Keep in mind that the “High” sample flow rate setting for tubes corresponds to about 60 µl / min. The minimum sample flow rate on the HTS is 30 µl / min (0.5 µl / s).