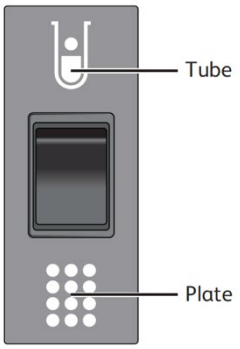
Imaging Center Essen	Institut für Experimentelle Immunologie und Bildgebung	
Standard **O**perating **P**rocedure **Workflow BD FACS A1 Symphony** Please note: If images acquired on IMCES instruments are used in publications we would be grateful if you could mention the facility in the Acknowledgements.		Last change: 18.11.2025

Using the High Troughput Sampler (HTS)

- With the High Throughput Sampler (HTS) you can automatically measure 96- and 384-multiwell plates
- We recommend to use **U-Bottom plates** (Corning, cat. no. 353910) for measurement of samples
- We recommend to use tubes for single stainings/FMOs and to set voltages for the experiment

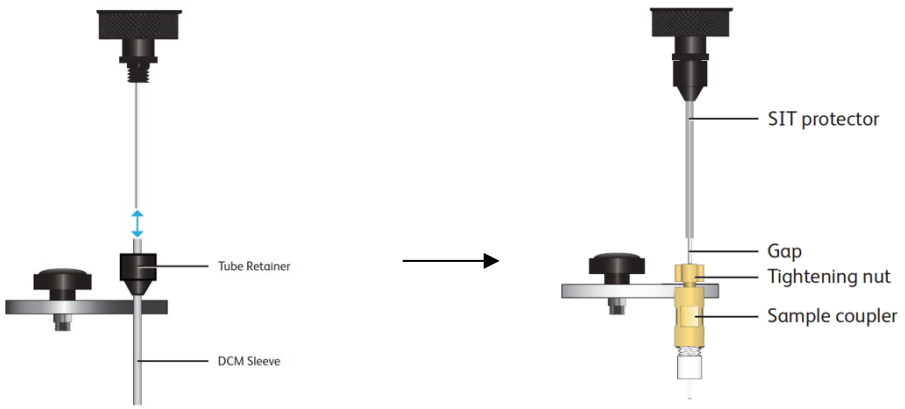
Starting Up the System

- Mostly the system start, initial cleaning, and if required CS&T, are done as as described in the “Normal” SOP for the BD FACSymphony A1
- Difference: Switch on the HTS unit as well (it is not recognized if you start the software before starting the HTS), set the acquisition control switch to plate mode (toggle switch under the green main power button)



Instrument Preparation

- These steps are to be done once you are done with setup/cleaning/calibrations in tube mode and want to proceed to measurements in plates
- Make sure that the water bottle on the shelf is full
- Put a glass beaker under the SIT
- Move the aspiration arm to the left and unscrew the tube retainer that holds the DCM sleeve onto the SIT and carefully remove the sleeve.
- Install the SIT protector (slide the protector over the SIT and push up on the tube retainer until you can screw it onto the SIT. Tighten the tube retainer, hand tight is enough.)
- Use your single stainings/FMOs to set voltages for the experiment
- Carefully attach the HTS coupler to the cytometer SIT. (Slide the sample coupler onto the SIT until you reach a hard stop. Make sure the sample coupler tubing is not kinked or twisted. Hold the coupler with one hand while you tighten the top nut with the other hand. There should be a gap between the tightening nut and the bottom of the SIT protector. If you don’t see a gap, unscrew the tube retainer, push the SIT protector all the way up and retighten the tube retainer. Hand tight is enough.)

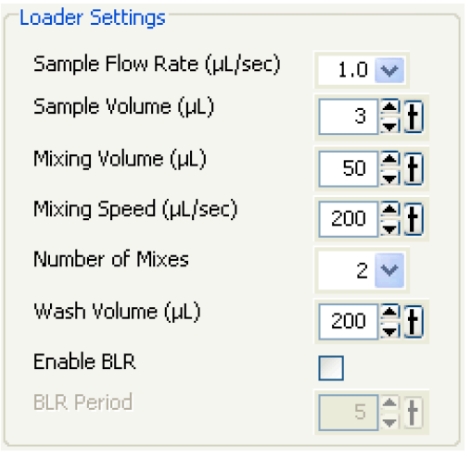


- Make sure the sample coupler is securely connected to the SIT.
- Make sure that the Symphony A1 is on RUN, flow rate coarse adjustment on LOW, and the fine adjustment is at 250
- Select “HTS” > “Reinitialize”
- Prime the HTS 2 times via the software

Setting Up a plate experiment

- Create a new experiment or load an experiment template
- Select “cytometer settings” and, if not done yet, select channels and gain voltages and create plots and gates as in tube mode
- Create a “new plate”
- Select the Troughput mode: we recommend to run the HTS in *Standard* mode

- Select your loader settings
- Sample flow rate 0.5 (≈30µl/min) to 1.0 (≈60µl/min)
- Sample volume: volume of your sample **minus 50µl** dead volume
- Mixing volume: not bigger than half the amunt of your sample
- Mixing speed and number of mixes: depends on your samples



- Create specimens and wells (make sure they are never named the same, will be overwritten and you have just the data of the latest recorded)
- Use the **Plate Controls** to acquire and record wells (if you do not see the Plate Controls, right-click into the Acquisition Dashboard window and activate them via the context menu)
- If you apply stopping criteria do this for your smallest population (the HTS will always inject the complete sample amount set, if the stopping criteria are reached before the leftover sample will go to the waste)
- Make sure you are in Global worksheet mode
- “Load” your plate, “record”
- Your event rate should be ≈2000 evts/sec and not higher than ≈5000 evts/sec (it’s recommended to dilute your samples instead of changing the flowrate)

Data Management

See “normal” FACSymphony SOP

After you finished:

Check the booking schedule at the end of your measurement

- **If there is a user after you:**

Standby

- Run IMCES Clean routine – 96 well U-bottom (4 wells Detergent Solution, 4 wells dH_2O, 4 wells FACS Clean, 4 wells dH_2O
- Disconnect the HTS sample coupler, remove the SIT protector and place back the DCM sleeve.
- Switch back to tube mode.
- Press Standby
- leave a FACS tube with dH_2O on the SIT
- Logout from the FACSDiva software

- **If you are the last user:**

Shutting down the system

- Run IMCES Clean routine – 96 well U-bottom (4 wells Detergent Solution, 4 wells dH_2O, 4 wells FACS Clean, 4 wells dH_2O
- Disconnect the HTS sample coupler, remove the SIT protector and place back the DCM sleeve.
- Switch back to tube mode.
- Press Standby
- leave a FACS tube with dH_2O on the SIT
- Logout from the FACSDiva software
- Shut down the computer
- Turn off the cytometer main power
- Shut down the FACSFlow Supply System