Standard Operating Procedures (SOPs): BD HTS (on Canto II)

General remarks

Following the introduction course and signing the Cell Analytics Facility policies are mandatory prerequisite to use the BD HTS on the FACSCanto II

Report any technical problems or questions to:

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I. Information

- HTS allows to acquire sample from 96 and 384 wells plates (standard depth)
- A 96-wells plate takes around 45min to be process.
- Each well can be mix before acquisition.
- Easy to switch between single tube and HTS mode.

II. Connect/Disconnect HTS

- To switch from tube to plate mode, attach the HTS sample coupler to the SIT (move the arm of the left, slide the sample coupler until your reach a hard stop, tighten the top nut).
- Then close the door and wait 2min for HTS to initialize.
- Home the HTS by choosing: "HTS→ Home"
- Prime the HTS twice by choosing: "HTS→ Prime"

- To disconnect the HTS, simply descrew the sample line from the SIT and close it again out of the SIT.

III. Acquisition

Note: Samples can be acquired in standard or high-throughput mode. High-throughput mode aspirates always 22ul.

Minimum volume per well is 50ul, Maximum is 200ul+20ul dead volume.

Maximum event rate is 10'000 events/sec.

- Switch on the BD FACSCanto II according to SOPs.
- Create a folder with your name.
- Create a new experiment or import a previous experiment template.
- Delete all non-needed parameters of the cytometer settings.
- Draw all needed graphs and prepare the worksheet.
- Click the "New plate" button in the browser toolbar.
- Click on the arrow next to the new plate to choose the type.
- Set-Up your plates design in the plate window:
    - Put the plate in STANDARD mode !!!
    - Select the different wells and add the Specimen Wells button. You can then change the specimen names in the inspector.

  - The loader settings can be modify in the plate window (mix volume = ½ sample volume)
  - Modify easily labels (specimen names and also fluorochrome labels) and number of cells using the "Experiment Layout"
- To set PMTs and also compensation controls, you have two methods:
  - TUBE = Create tubes, pass your sample and set PMTs. Then choose "Experiment → Compensation Setup → Create Compensation Controls". Acquire your single settings and "Create Compensation Matrix".
  - PLATE = Add setup controls wells to the experiment (used for unstained controls to set PMTs). Select the different setup controls wells and click on RUN WELLS. Adjust FSC, SSC and PMTs using the unstained control. Then select a well and choose "Experiment → Compensation Setup → Create Compensation Controls". The specific compensation controls will be added in the next wells. Continue with the compensation by selecting all compensation wells and click on RUN WELLS. At the end, verify P1 gate and calculate compensation.
- Put the plate on the HTS. Make sure that the A1 well is at the back-right corner of the stage. Close the door.
- **Then select the first well from the experiment** (must be green) and RUN PLATE.

IV. Cleaning

Note: The cleaning process is essential to avoid clogging or contamination of the HTS.

- The instrument must be cleaned between every user. If you are running problematic samples, you might have to clean in between your experiments to avoid clogging.

- **Choose "HTS →Clean →Daily clean"**
- **Prepare a 96Well plate with 150ul DI Water in A1-A4, 150ul FACSClean in B1-B4, 150ul FACSRinse in C1-C4 and 200ul DI Water in D1-D4**
- **Place the plate on the HTS with the A1 well on the back-right corner of the stage.**
- **Close the door.**
- **Click OK on the cleaning confirmation message and wait 15min.**
- **Click OK when the completion message appears.**

- Keep the HTS connected to the SIT arm all the time.

V. Shutdown

- Check on Open IRIS if somebody is booked after you: http://iris.science-it.ch

- *Between different users of the day:*
  - Logout the FACSDiva Software.
  - Leave instrument and computer running for the next user.
  - Clean the work area - don't leave used tubes, gloves, and etc. behind.

- *Last user of the day:*
  - Run the "fluidics shutdown " procedure on "Cytometer" (take 10min, keep the HTS connected to the SIT))
  - Close the FACSDiva Software.
  - Shut down the computer.
  - Turn off the instrument by pressing the green button on the left side of the Canto II
  - Clean the work area – don't leave used tubes, gloves, and etc. behind.