BD FACS CantoII Analyzer
HTS Plate Loader SOP

A – Introduction

The BD High Throughput Sampler (HTS) is a high-speed sampling device connected to the BD FACS CantoII analyzer to enable sample acquisition from 96 well plates. This document serves as a step-by-step process of startup, setup, acquisition, cleaning and shutdown of the HTS system.

ALL USERS MUST BE TRAINED ON THE USE OF HTS BY BFC STAFF PRIOR TO USE.

B – Startup of the HTS

1. Switch ON the power button of the HTS module (on the right side of the BD FACS CantoII analyzer).
    a. *If the analyzer has already been in use, you need to restart the system: quit FACSDIVA, shutdown the PC and power off the analyzer. Switch on the HTS, then proceed with turning on analyzer, computer and FACSDiva.*
2. **Carefully** attach the sample coupler to the SIP. Loosen a bit the top nut of the sample coupler and push it onto the SIP until you feel a stop. Hold the sample coupler in place and tighten the top nut (see Figure 1).

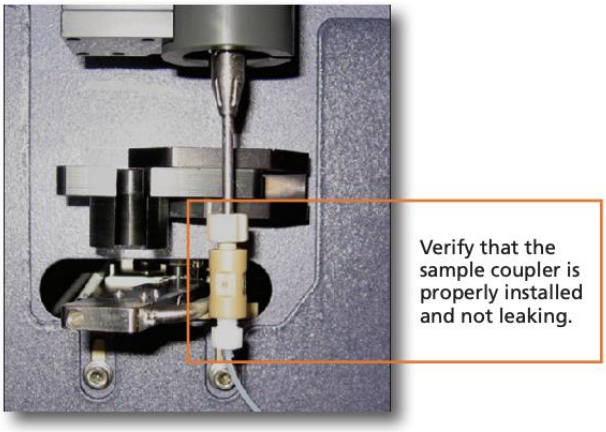

Figure 1 - Sample Coupler connected to the SIP

3. Close both HTS doors, and prime the HTS by selecting Menu **HTS > Prime** on FACSDiva. Repeat the priming 3 times to effectively remove all air bubbles in the HTS tubing.
4. Prepare the cleaning plate for the HTS Pre-Clean:
    a. Add 250 µl of BD FACS Clean to wells A1-A6
    b. Add 250 µl of BD FACS Rinse to wells B1 – B6
    c. Add 250 µl of MilliQ water to wells C1 – C6
5. Load the plate in the HTS plate stage and close both the HTS doors. Well A1 should go on the back-right corner of the plate stage (see **Figure 3**).
6. Open a HTS Cleaning Experiment by selecting **Experiment > New Experiment** and choosing **HTS Cleaning Experiment** on the General tab in the Template Window. Perform the HTS Pre-Clean by acquiring wells A1-A6, B1-B6, C1-C6 at max flow rate (3.0 µl/sec, 200 µl sample volume, stopping criteria set on gate P1 of Cleaning Worksheet for 10,000,000 events).

C – Setting Up and Running an Experiment

1. Create a new experiment on FACSDiva (see **CantoII SOP, Section D, E and F**).
2. In the **Browser** toolbar, select the plate type used in the drop-down menu (see **Figure 2**). A Plate Layout Editor should appear (or double-click on the plate name in the Browser to view it).

Figure 2 - Plate icon on the workspace toolbar

3. In the **Plate Layout Editor** select with the mouse all the wells containing samples. Maintaining the selection, click on the **Specimen** icon on the Plate Layout Editor to create a new specimen containing the wells to be sampled.
4. Select the specimen(s) and setup the instrument settings for the specimen acquisition:
    a. **Plate information**: select either High or Standard throughput.
        i. *Note: with High throughput selected, only 22 µl of the sample will be analysed.*
    b. **Sample Flow Rate**: from 0.5 µl/sec to 3.0 µl/sec. *We recommend setting a flow rate of 1.0-1.5 µl/sec for sample analysis. Higher flow rates in samples containing cells and/or beads will likely clog the HTS.*
    c. **Sample Volume**: is the amount of sample to be analysed. When setting the sample volume remember that the HTS aspirates 20 µl extra volume (not analysed) and leaves 25 µl of dead volume in the well. So, for a sample of 245 µl, the maximum sample volume that can be analysed is 245 – 20 – 25 = 200 µl.
    d. **Mixing volume**: set the amount of sample volume mixed before its acquisition. Recommended value: half the total volume of the sample, in µl. For example, if the sample contains 245 µl, set the mixing volume to 120 µl.
    e. **Mixing speed**: set the speed of the sample agitation before its acquisition. Recommended values: 180-200 µl/sec.
    f. **Number of mixes**: set the number of mixes before the sample acquisition. Recommended value: 2.
    g. **Wash volume**: set the volume of FACSFlow sheath fluid used to wash the HTS fluidics in-between samples. Recommended value: 400 µl.

    More information about the plate loader settings can be found in the **Appendix, Section 1**.

5. Set up the stopping criteria for recording the samples by selecting **Experiment > Experiment Layout** and clicking on the **Acquisition** tab. Select all the samples to be recorded using the mouse and the Shift command. Apply the Global

Worksheet to be used for the sample analysis and set the **Stopping Gate**, **Number of Events to Record** and **Storage Gate**.

a. *We recommend setting the Storage Gate always on All Events, with the exception of the LegendPlex/CBA assays, in which you should choose the gate identifying all the beads.*

6. Load the plate on the HTS plate stage. Well A1 should go on the back-right corner of the plate stage (see **Figure 3**). Close both HTS doors.

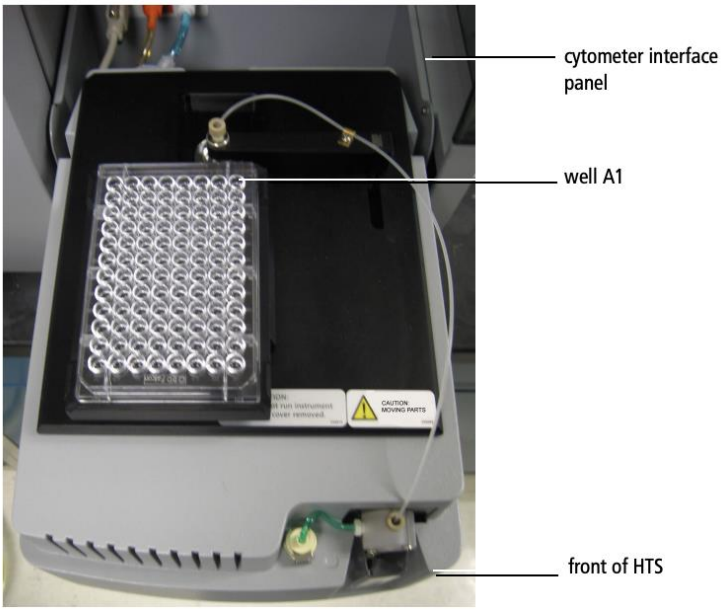

Figure 3 - Plate oriented and loaded onto the HTS

7. To acquire and record 1 sample, select its well on the Plate Layout Editor and click on **Run Well(s)** in the **Acquisition Dashboard**. You can also record a selection of wells using the same command.
8. To acquire and record the full plate, click on **Run Plate** in the **Acquisition Dashboard**.
    a. ***Note – Pausing a sample and/or plate acquisition:*** *if one pauses or stops the acquisition (standard mode) the current well will be lost. If one pauses or stops the acquisition (high throughput mode) the current well and the next well will be lost.*
9. At the end of the run, a dialogue message appears to indicate that the acquisition is complete.

D – Exporting Data

Please consult the BD FACS CantoII Analyzer SOP (Section G – Exporting Data) to export your experiment data.

E – Post-Run Cleaning

1. Prepare the cleaning plate for the HTS Post-Clean:
    a. Add 250 µl of BD FACS Clean to wells F1-F6
    b. Add 250 µl of BD FACS Rinse to wells G1 – G6
    c. Add 250 µl of MilliQ water to wells H1 – H6
2. Load the plate in the HTS plate stage and close both the HTS doors.
3. Open the HTS Cleaning Experiment. Perform the HTS Post-Clean by acquiring wells F1-F6, G1-G6, H1-H6 at max flow rate (3.0 µl/sec, 200 µl sample volume, stopping criteria set on gate P1 of Cleaning Worksheet for 10,000,000 events).
4. Remove the plate, and prime the HTS by selecting Menu **HTS > Prime** on FACSDiva. Repeat the priming 3 times to effectively remove all air bubbles in the HTS tubing.
5. Disconnect the sample coupler from the SIP, by loosening the top nut and gently pulling the sample coupler off the SIP.
6. If another user is booked after you in the same day, log out from FACSDiva by selecting **File > Log Out** and choosing **Log out without fluidics shutdown** when prompted by the dialogue box.
   If no one else is booked after you, perform a Shutdown (See **BD FACS CantoII Analyzer SOP, Section I - Shutting Down the System**).

APPENDIX

1 – Plate Loader Settings

Loader Setting	Description	Important Considerations
Sample Flow Rate	Amount of sample (in µL per second) that is delivered to the flow cell. Select a rate between 0.5 and 3.0 in increments of 0.5 µL per second.	The larger the value entered, the shorter the plate running time, but this increases the sample core, causing more variation of data.
Sample Volume	Amount of sample (in µL) aspirated from the well and delivered to the flow cell. Select a volume between 2 and 200 µL.	For High Throughput mode, the system aspirates a set amount of 22 µL of sample, but records data for a volume between 2 and 10 µL. For Standard Throughput mode, the system aspirates the sample volume amount plus 20 µL. This value does not include the plate-dependent dead volume. Larger volumes can increase run time.
Mixing Volume	Amount of sample (in µL) aspirated and dispensed from the well to resuspend the particles.	To avoid introducing bubbles into the fluidics, this value should be half the total well volume.
Mixing Speed	Rate (in µL per second) that the mixing volume sample is aspirated and dispensed.	The faster the rate, the more likely that cell shearing occurs, especially for delicate cells. A faster rate can introduce bubbles in the sample delivered to the cytometer and compromise the separator bubble.
Number of Mixes	The number of times the mixing volume sample is aspirated and dispensed at the mixing speed. Select a number between 0 and 5 mixes.	The larger the number, the longer the plate running time.
Wash Volume	Amount of sheath fluid (in µL) drawn through the HTS fluidics between wells. Select a volume between 200 and 800 µL.	Enter a higher value to reduce cross contamination between wells. Enter a lower value to decrease the plate running time.

2 – Further Considerations

1. The HTS is prone to clogging. To avoid clogs, the cell concentration in the sample should not exceed 6 x 10^6 cells/ml in 250 µl volume (= max 1.5 x 10^6/well). Always filter the samples using a 70 µm cell strainer and resuspend the samples by pipetting up & down with a multichannel pipette before loading on the HTS (avoid creating bubbles).

2. Software will stop acquisition (recording of a well) and proceed to the next well when either the specified number of events set in the stopping criteria is reached or the stopping time is reached. Stop time (sec) = sample volume (µl) divided by flow rate (µl/sec). Set number of events high if you want to record up to the stop time.

3. Do not exceed a 1x 10^6 cell count per well in the acquisition field of the FACSDiva program (located in the acquisition layout). This will result in a plate memory error.

3 – Troubleshooting

3.1 – Low number of events acquired

- Possible sample leaks: check the connection between the sample coupler and the SIP to exclude leaks. In the case of incorrect connections, remove the sample coupler and repeat the connection procedure.
- Incorrect setup of the stopping conditions for recording: check that the stopping criteria for sample recording have been set up correctly in the Experiment Layout, Acquisition tab.
- Bubbles in sample wells:
    - inspect the plate to check for bubble presence and tap the plate to remove bubbles.
    - Check that the mixing volume is not exceeding half the total sample volume.
    - Prime the HTS
- Contact BFC Staff for further troubleshooting.

3.2 – No events shown on the plots or population shifting in the FSC vs SSC plot

- No events displayed or shifts in the scatterplot usually indicates a partial or complete clog. Stop the plate acquisition and run a Post-Clean (**Section E, 1-3**) to de-clog the instrument.
- Consider resuspending your samples with a multichannel or filtering them again.
- Contact the BFC Staff for further troubleshooting.

3.3 – Unavailable Run commands in the Acquisition Dashboard

- Select the Global Worksheet to be used for the analysis in the Experiment Layout, Acquisition Tab. Apply to all samples to be recorded.
- For Run Well(s): select the well(s) to be recorded.