BD FACSymphony™ A1 with HTS – User's Guide

For authorization to use the FACSymphony cytometer, you have to receive a training and must accept the user rules of the facility!

Tube-based Acquisition

Cytometer Startup

1. Switch on FFSS (FACSFlow Supply System).
2. Switch on computer.
3. Switch on cytometer.
4. Remove sample coupler from SIT, if connected:
5. Install a tube with 1 ml (not more!) of DI water on the SIT.
6. Press Prime in the Fluidic Controls > air bubbles are visible in the water tube.
7. Press Run in the Fluidic Controls.
8. Set Flow Rate to Low.
9. In Windows log in as BDOperator > Password: Welcome#1
10. Launch Diva.
11. Select your account and log in.

Data Acquisition

1. Create a new experiment in the browser.
2. Select the detectors you want to use in the cytometer settings window and delete non-used ones.
3. Create a new specimen and activate the first tube with the tube pointer.
4. Create plots on worksheet and define axis labels. Open Population Hierarchy and Statistics View with right-click on plot. Right-click on Statistics View to edit.
5. Move aspirator arm to the left and install the first sample tube on the SIT. Please note: If cytometer is in Run mode sample is continuously consumed!
6. Click Acquire Data in the Acquisition Dashboard.
7. Adjust PMT voltages in the Cytometer Settings window.
8. Define gates.
9. Define number of events to record, stopping gate, etc.
10. Click Record Data in the Acquisition Dashboard.
11. Remove sample tube from SIT and install the next sample.

Exporting Data

- To export FCS files, go to File > Export > FCS
- To create a PDF file right-click the experiment > Batch Analysis > select Save as PDF and define storage location (USB stick) > start
- To create a CSV file based on the statistics selected in the Statistics View right-click the experiment > Batch Analysis > select Save Statistics and define storage location (USB stick) > start

Cleaning

1. Install a tube with 1 ml FACS Clean. Let it run on High for 3 minutes.
2. Install a tube with 1 ml DI Water. Let it run on High for 3 minutes.
3. Install a tube with 1 ml (not more!) of DI water on the SIT and set cytometer on Standby.

Cytometer Shutdown

1. Switch off cytometer.
2. Switch off FFSS.
3. Log out from Diva and shutdown computer.

Tank maintenance

1. Switch off alarm sound on FFSS.
2. Empty the Waste Tank and/or replace the Sheath Tank.
3. Press Restart on FFSS.
4. If air bubbles are visible vent sheath filter.

Plate-based Acquisition (96-well or 384-well)

Cytometer Startup

1. Switch on FFSS (FACSFlow Supply System).
2. Switch on computer.
3. Switch on cytometer.
4. Make sure that the sample coupler is installed on the SIT.
5. Make sure that the Tube/Plate Acquisition mode switch on the cytometer is in Plate mode.
6. Make sure that the HTS is switched on and the HTS cover is installed.
7. Press Run in the Fluidic Controls.
8. In Windows log in as BDOperator > Password: Welcome#1
9. Launch Diva.
10. Select your account and log in.

Data Acquisition

1. Create a new experiment in the browser.
2. Select the detectors you want to use in the cytometer settings window and delete non-used ones.
3. Create a new specimen and activate the first tube with the tube pointer.
4. Create plots on worksheet and define axis labels. Open Population Hierarchy and Statistics View with right-click on plot. Right-click on Statistics View to edit.
5. Open a new plate in the browser. Make sure to select the correct plate type.
6. In the plate layout define 1-2 setup wells.
7. Click Run Wells in the Acquisition Dashboard.
8. Adjust PMT voltages in the cytometer settings window.
9. Define gates.
10. In the plate layout define specimen wells.
11. Select the wells you want to record. Choose sampling mode and adjust loader settings if needed.
12. Define number of events to record, stopping gate, etc. for each well using either the Acquisition Dashboard or go to Experiment > Experiment Layout.
13. Click Run Plate.

Exporting Data

- To export FCS files, go to File > Export > FCS
- To create a PDF file right-click the experiment > Batch Analysis > select Save as PDF and define storage location (USB stick) > start
- To create a CSV file based on the statistics selected in the Statistics View right-click the experiment > Batch Analysis > select Save Statistics and define storage location (USB stick) > start

Cleaning

1. Install a plate with 200 µl FACS Clean in well A1-A4 and 200 µl DI Water in well B1-B4.
2. HTS > Clean.

Cytometer Shutdown

4. Switch off cytometer.
5. Switch off FFSS.
6. Log out from Diva and shutdown computer.

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Tank maintenance

5. Switch off alarm sound on FFSS.
6. Empty the Waste Tank and/or replace the Sheath Tank.
7. Press Restart on FFSS.
8. If air bubbles are visible vent sheath filter.

HTS Specifications

	Standard Mode		**High Throughput Mode**	
	Default	Range	Default	Range
Well volume 96 well	250 µl	50-300 µl	100 µl	50-300 µl
Well volume 384 well	50 µl	50-120 µl	50 µl	50-120 µl
Aspirated volume	sample volume + 20 µl		fixed volume = 22 µl	
Sample volume	10 µl	2-200 µl	2 µl	2-10 µl
Sample flow rate	1 µl/s	0.5-3.0 µl/s	1 µl/s	0.5-3.0 µl/s
Sample flow rate	60 µl/min	30-180 µl/min	60 µl/min	30-180 µl/min
Mixing volume	one-half the available volume			
Mixing speed	180 µl/s	25-250 µl/s	200 µl/s	25-250 µl/s
Number of mixes	2	0-5	2	0-5
Wash volume	400 µl	200-800 µl	200 µl	200-800 µl
Stopping time 1 well	sample volume (µl)/sample flow rate (µl/s)			
Max event rate	35000 evt/s			