

BD High Throughput Sampler User's Guide

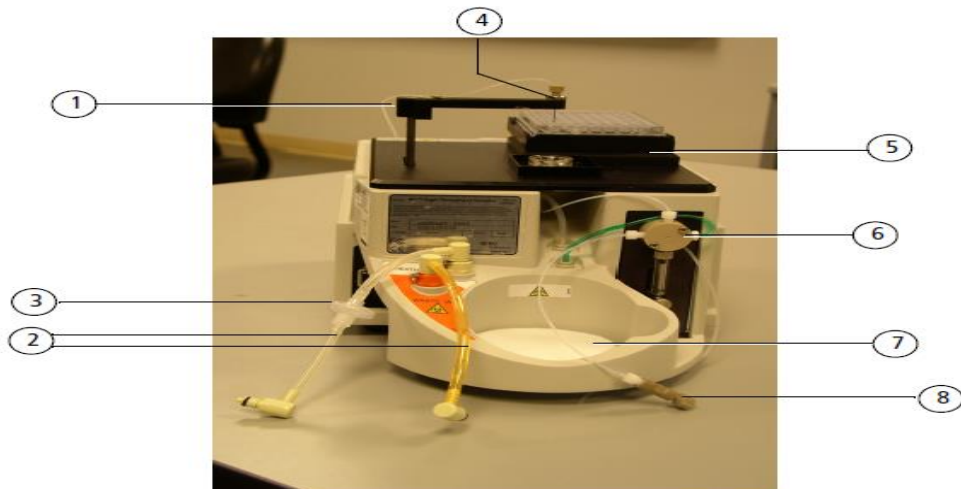
For the

BD LSRFortessa

I. Introduction


The BD High Throughput Sampler (HTS) is a high-speed sampling device used on the 5-Laser LSRFortessa located in Rm. 6006F of the RIA Division. The HTS provides users the ability to acquire samples from 96-well or 384-well plates for efficient analysis with minimal carryover. This guide will serve as a step-by-step process of installation, acquisition, shutdown and removal of the HTS system. ALL USERS MUST BE TRAINED PRIOR TO USE.

NOTE: When booking time on the calendar please account for at least 30-45mins total time of setup & cleaning/breakdown of the HTS.



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| ① | Probe assembly—moves front to back and up and down to transfer sample between plate holder and injection port/wash station |
| ② | Fluidics tubing: sheath (clear) and waste (orange) |
| ③ | Sheath filter—filters incoming sheath fluid to HTS unit |
| ④ | Injection port/wash station—provides interface for sample injection and probe washing |
| ⑤ | Plate holder—moves left to right and front to back to position plate so the probe can pick up sample |
| ⑥ | Secondary pump and valve—delivers sample to flow cell in high-throughput mode |
| ⑦ | Absorbent pad—collects potential overflow from the injection port/wash station or drips from the cytometer sample injection tube (SIT) |
| ⑧ | Sample coupler—connector between HTS unit to cytometer SIT |

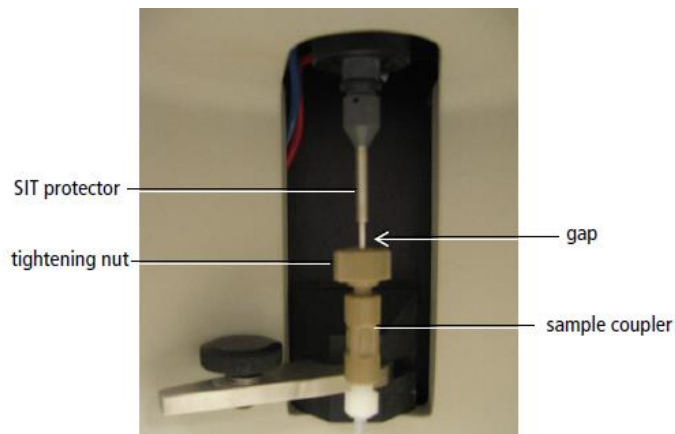
II. Installation of HTS for Plate-Based Acquisition. (Note: The devices are HEAVY and may require some assistance from Core Personnel)

1. Make sure sheath tank is full and waste is empty.
2. Prime & Setup machine as normal.
3. Run compensation controls as tubes.
4. Prior to moving HTS devices: Switch acquisition control switch to Plate mode located to the right of the sample station with instrument in “standby”.  (When Plate mode is on the aspirator pump is off, if instrument is in “run” the SIP will drip)
5. Move aspirator arm to the side and remove Droplet Containment Sleeve (DCM). Unscrew (clockwise) the tube retainer until you can remove the DCM sleeve freely. Store DCM sleeve in styrofoam rack.
6. Replace DCM with SIT protector which is kept in the drip tray on HTS unit (See Figures Below)



7. Move tubing and wires connected to Fortessa under the sample port for easy access.
8. Remove HTS safety cover and install HTS Unit onto the cytometer. HTS Unit base plate is permanently installed on the Fortessa. HTS Unit is placed on the sliding base plate to the right of the sample station. It will be easier to attach power and communication cables with HTS unit facing the user.
9. Connect the power and communication cables to ports on the side of the HTS facing the wall. **NOTE: DO NOT GET THE POWER CORD WET. IT WILL SHORT CIRCUIT THE ENTIRE INSTRUMENT.**

10. Once power and communication cords are connected, slide the HTS Unit in to place with absorbent pad underneath the SIP.
11. Locate the “plunger” and bleed sheath tubing connected to cytometer.
12. Disconnect “purging assembly line” from the HTS and place off to the side for use in Step 1 of removal.
13. Place a few drops of sheath fluid from sheath dropper in the now empty sheath connector on HTS prior to connecting lines.
14. Connect the sheath and waste line to the HTS unit.
15. CAREFULLY, attach the sample coupler to the SIP (Sample Injection Probe). Push sample coupler onto the SIP until you feel a stop. Hold the sample coupler in place and tighten the top nut. (See Figure Below)



16. Place safety cover back on HTS Unit and turn on the HTS power switch located near the power cord connection on the side of the machine facing the wall.
17. Set Instrument to “Run” and open BD FACSDiva software.
18. In BD FACSDiva software, select HTS > Prime, 2-3x to remove air bubbles caught in tubing. (Note: Safety cover must be on for software to execute Prime function. Inspect blue/green tubing connected to the syringes/pumps on the front and back of the HTS unit where air is usually seen. Tap tubing in between primes to loosen any air bubbles caught in tubes. It will be necessary to remove the safety cover to access the tubing on the front syringe/pump, tap tubing then replace cover before priming. Many Primes may be necessary in order to completely remove all air)

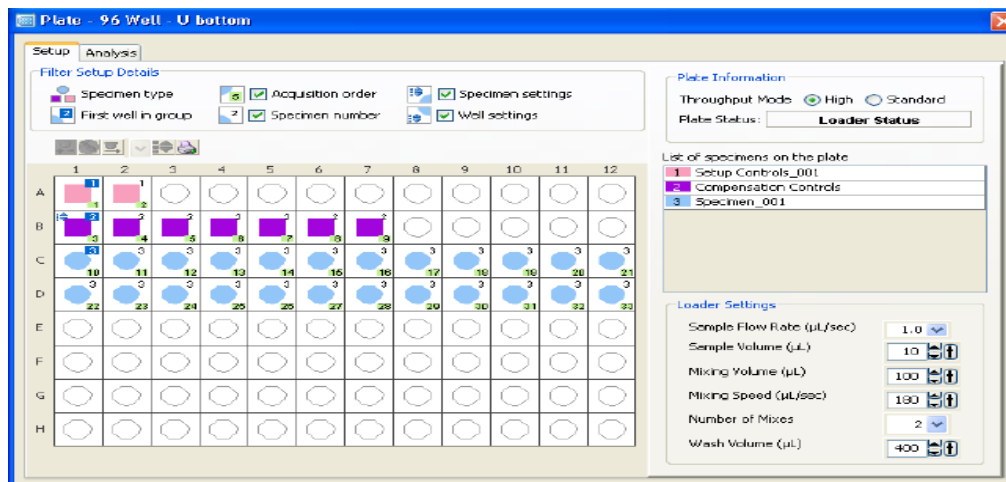
- NOTE: If Diva is already running, install HTS device and select “ReInitialize” from HTS drop-down menu. Communication between HTS unit and software will now be connected.


III. HTS Acquisition

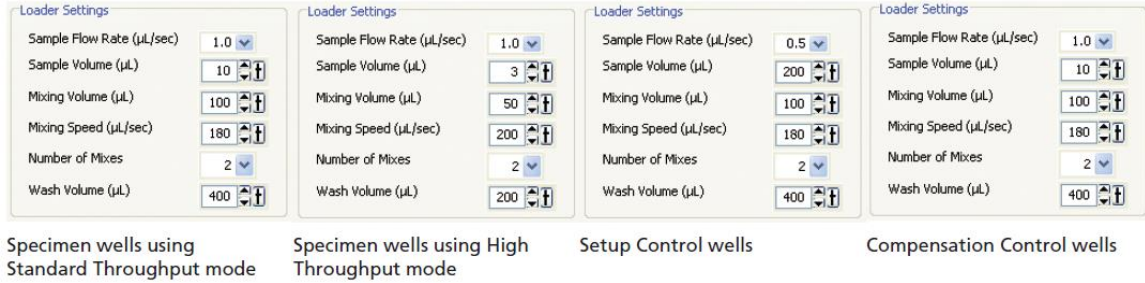
Note: Run compensation and setup controls as tubes, prior to running plates.

Setting up Experimental Plate Editor and Worksheet:

- Create a new experiment in the browser window and select the parameters used in the experiment.
- In the browser toolbar select the type of plate used in the pull down menu next to the plate icon. Plate layout should appear. If not, double-click created plate icon in browser window to view layout editor. (See Figure Below)



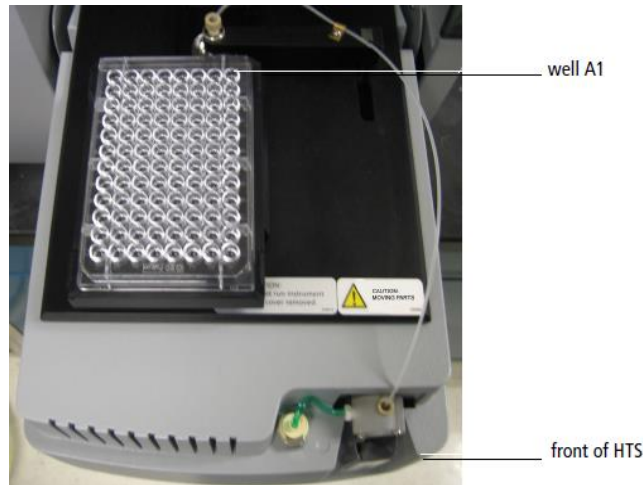
- In the plate layout window, select the wells that have experimental samples by either holding the Shift key to select continuous wells or the Control key to select individual wells. Select the Specimen icon to apply specimen wells.  This will assign each selected well with a specimen and applies default settings for acquisition accordingly.
- Select the Throughput Speed for Specimen tubes. Default settings are applied with respect to Standard/High Throughput speeds. (Note: These settings can be modified depending on sample volume and concentration.) (See Figure Below)



- Note: Plate acquisition can only be recorded using a Global Worksheet. View the Unstained Normal Worksheet, and choose Edit > Select All > Copy. Then view the global worksheet, right-click and choose Paste.
- Layout setup is complete and samples are ready to be acquired. (Note: If running multiple plates under the same setup and compensation conditions, create one layout for each plate that includes the proper setup/compensation/specimen wells)

Running Plate:

- Remove safety cover from the HTS unit and place the plate with well A1 aligned with marking on the HTS stage (See Figure Below)



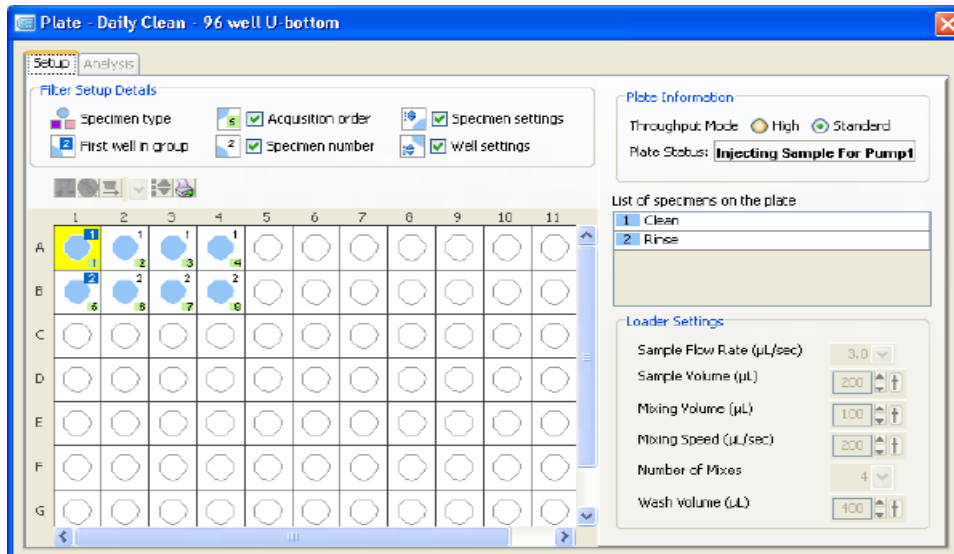
- Replace HTS safety cover.
- On the Acquisition Dashboard, right-click and select Show Plate Controls.
- IMPORTANT:** Setting Events to Record: Either click each well separately and select desired events, or go to Experiment > Experiment Layout. Under the Acquisition tab, select wells and assign number of events to record. (Note: In the plate editor window only the green highlighted well is changed when selecting

events to record even if multiple wells are selected. If sample is loaded and not collected, it is LOST)

5. Select specimen well and click Run Wells on the Acquisition Dashboard
6. Once plate has finished running, a second plate can be loaded on to HTS with the correct plate layout selected or move on to the cleaning/shutdown procedure.

IV. Cleaning/Shutdown Procedures

1. A separate plate must be created with 8 wells: (See Figure Below)
 - a. A1-A4 = 4 wells Coulter Clenz
 - b. B1-B4 = 4 wells DI Water

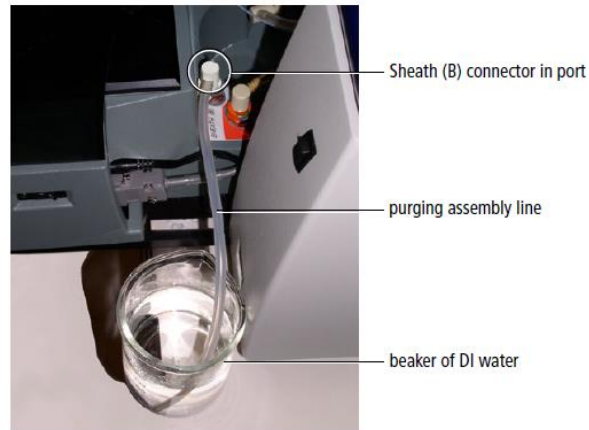


2. Remove Cover on HTS and place Cleaning Plate in same orientation as previous experimental plates.
3. HTS > Clean, select Daily Clean
4. Replace cover and click OK
5. Once finished daily cleaning procedure, move on to Removing HTS

V. Removing HTS Unit and Set Up for Tube-Based Acquisitions

1. Disconnect sheath line from the HTS and tuck under Fortessa and connect the “purging assembly line” that was disconnected from Step 12 of Installation to the

HTS in its place. Place the open end of tube in a beaker of H₂O. (See figure below)



2. HTS > Prime 3x
3. Place cytometer in “Standby”
4. Leave “purging assembly line” attached to HTS.
5. Disconnect the waste line from the cytometer
6. Detach the sample coupler from the SIP
7. Disconnect the power and communication cables from the unit. Cover power cord adapter with a clean, dry tube.
8. Carefully lift and remove the unit.
9. Remove SIT and place in absorbent tray on HTS Unit
10. Replace the DCM sleeve
11. Tuck all cords attached to the cytometer off to the side out of the way of the sample chamber
12. Switch acquisition control switch to tube mode.

IMPORTANT!

VI. Additional Procedural Notes and limitations:

1. Cytometer must be placed in “Run” and cover installed on HTS prior to opening BD FACSDiva software in order for it to be recognized by the computer.
2. If Diva is already running, install HTS device and select “ReInitialize” from HTS drop-down menu. Communication between HTS unit and software will now be connected.
3. Cytometer can be placed in “Standby” when not running plates, primes, cleans, etc.
4. Cell concentration should not exceed 6×10^6 cells/ml in 250 μ l volume.
5. Software will stop acquisition (recording of a well) and proceed to the next well when either the specified number of events collected is reached or the stopping time is reached. Stop time (sec) = sample volume (μ l) divided by flow rate (μ l/sec). Set number of events high if you want to record up to the stop time.
6. Plate should be mixed prior to HTS setup. Avoid creating any air bubbles in well.
7. Use only plates made by either BD Falcon or Costar. Only these plates are validated for the HTS.
8. Do **not exceed** a 1M cell count per well in the acquisition field of the Diva program (location in the acquisition layout). This will result in a plate memory error.