

FACSFortessa™ – HTS Programing

The BD™ High Throughput Sampler (HTS) option for the BD LSRFortessa™ cell analyzer provides rapid, fully automated sample acquisition from microtiter plates. In high-throughput mode, the HTS can process a 96-well plate in fewer than 15 minutes. Standard throughput mode can be selected for acquisition of larger sample volumes.

Experiment Set-Up

- Create a new experiment: Folder icon OR Experiment → New Experiment.
- For parameter adjustment add ONE tube. Hit the Current Tube Pointer until it turns green.
- Go to Cytometer Window → Parameters → Add/delete fluorophores you do (not) need. These settings will be applied to all your samples on the plate!
- Create your worksheet with all necessary plots.
- Click the arrow next to 'New Plate' button in Browser window, choose between different plate formats. Plate window opens in Setup view.
- Design your experiment by defining your wells.



- Pink square = Setup controls
- Blue circle = Specimen
- Purple square = Compensation controls

Setup Controls

For adjusting PMT and gates. Note: Samples will not be recorded when you choose the option Run plate/wells. Click Record button for saving your setup controls.

Compensation Controls

The respective fluorochromes you have selected before will automatically appear in your plate layout.

Specimen

Please define only ONE specimen including all your samples to reduce file size.

Select the respective specimen in the list and do your settings. Deviations between individual samples will be encircled in red.

- ☑ In the Experiment layout define events to record and stopping gates for your specimen or single wells. Experiment → Experiment Layout → Acquisition tab

Run Control Samples

Setup Controls

- ☑ Select wells, click Acquire (+Record data) in Acquisition dashboard, NOT Run wells.
- ☑ Adjust PMTs and add gates as needed, click Restart when necessary.

Compensation controls

- ☑ Double-click onto Global Worksheet icon, single sheets appear for every compensation control.
- ☑ Select unstained control well. Click Acquire and adjust PMTV, click Record.
- ☑ Adjust P1 gate, right-click onto gate → Apply to all.
- ☑ Select all other single stained controls, click Run wells in Acquisition dashboard.
- ☑ Calculate compensation by Experiment → Compensation Setup → Calculate Compensation. Choose Link&Save.

Note: Don't change PMTV of fluorescence channels after enabling compensation.

Run your Specimen

- ☑ Enable compensation: Cytometer window → Compensation → Enable compensation.
- ☑ Go back to global worksheet.
- ☑ Select specimen wells, click Run wells in Acquisition dashboard.
- ☑ Recorded wells will be displayed as a disk.

Stopping the Assay

- ☑ If there are any errors occurring, the respective well will be colored differently.
- ☑ The run can be paused manually by clicking Pause/Stop/Resume.
- ☑ Stopping a run: In standard mode the current well will be lost, in HTS mode the current AND next well will be lost.

Analysis and Display of Data

- In Plate window, got to Analysis mode, click individual wells and data will be displayed in your worksheet.
- For batch analysis, go to Analysis mode on Plate window, select all wells, right-click and choose batch analysis.

Cleaning Procedure after Measurement

- Prepare a 96well-plate with each 4 wells of Clean -Rinse - MQ H2O
- Note: You can also include the cleaning wells already on your plate.
- Run cleaning procedure: HTS → Cleaning procedure → Follow instructions.

Shutting Down the System

Return to Tube-based acquisition mode:

- Re-connect the water to the HTS sheath port.
- Prime: HTS → Prime 3x.
- STNDBY mode.
- Switch off HTS.
- Re-install the standard DCM sleeve.
- Switch to Tube mode.
- Prime 3x with arm at side.
- Install MQ-water tube, close aspirator arm.
- Close open experiments, close the DIVA software, turn off instrument, fluidic cart and PC.