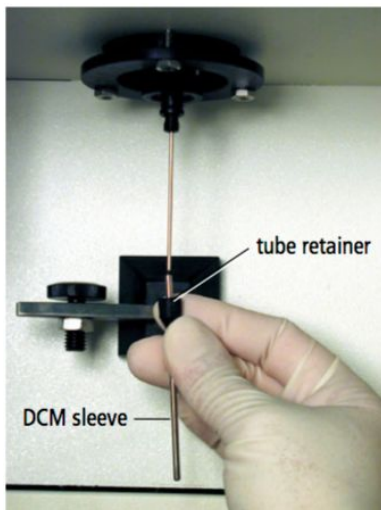


Start Up

Empty Waste container and fill Sheath tank
Turn on the Fortessa
Run through cleaning as per original training
Check the instrument QC

Switch from Tube-based to Plate-based Acquisition

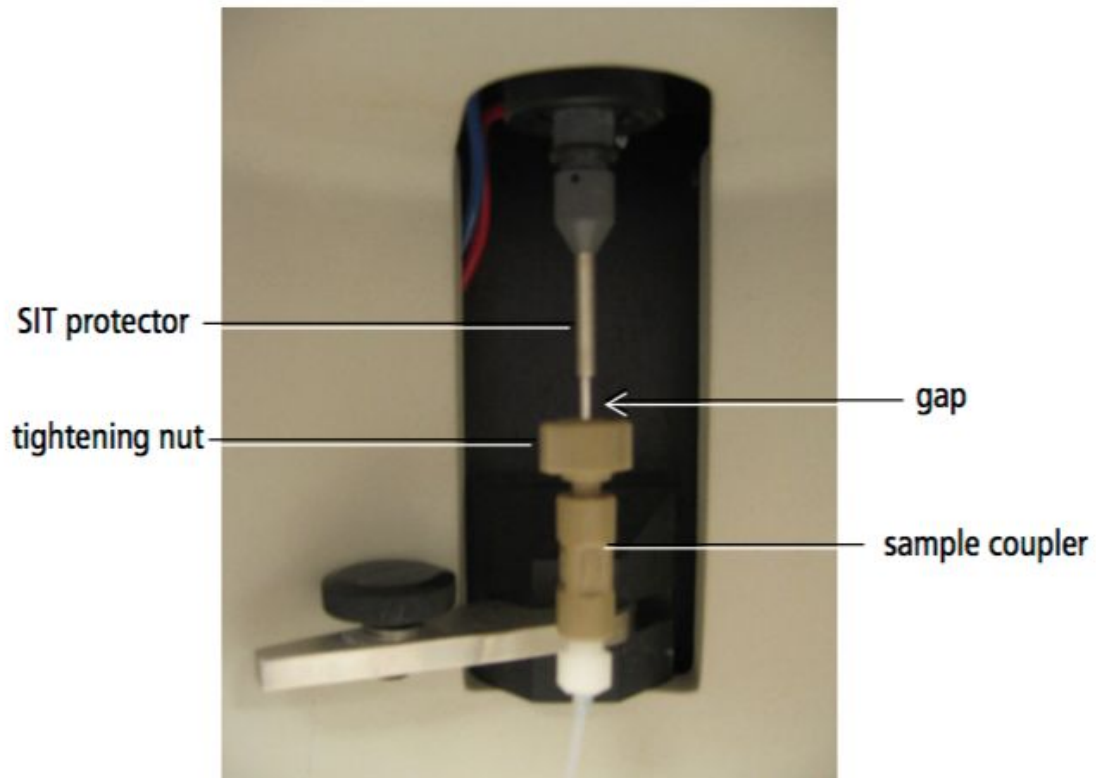
- 1) Set the acquisition control switch FROM tube mode (U) TO plate mode (⊙). The switch is on the Fortessa's right-side panel near the green ON/OFF button.
- 2) Move the aspirator arm to the left.
- 3) Unscrew the tube retainer that holds the DCM (droplet control module) sleeve onto the SIT and CAREFULLY remove the sleeve as in the pictures below:



- 4) Install the SIT protector.
 - a) Slide the protector over the SIT and push up on the tube retainer until you can screw it onto the SIT.
 - b) Tighten the tube retainer.



- 5) **CAREFULLY** attach the HTS sample coupler to the cytometer SIT (*do not bend the SIT*) as described here:
- Slide the sampler coupler onto the SIT until you reach a hard stop. Make sure the sample coupler tubing is not kinked or twisted.
 - Hold the coupler with one hand while you tighten the top nut with the other hand.
 - There should be a gap between the tightening nut and the bottom of the SIT protector (see illustration below). If you do not see a gap, unscrew the tube retainer, push the SIT protector all the way up, and retighten the tube retainer.
 - Make sure the sample coupler is securely connected to the SIT.



- Turn on the HTS (power switch is in the back of the HTS).
- Place the cytometer in **RUN** mode.

- 8) After the initialization sequence has finished, select **HTS > PRIME**.
- 9) Run the HTS prime 2 to 3 times.

Plate Setup and Data Acquisition

- 1) Click New Plate and choose the plate type.
- 2) Use the Plate window to select the throughput mode (standard mode is recommended).
A maximum of 10 μ L of sample is acquired in High Throughput mode.
- 3) Plate Set-up:
 - i. Setup Controls (unstained cells) - recommended at least two wells of unstained cells to set all setting (no data is saved)
 - ii. Compensation wells – Select well > Experiment > Compensation setup > Create compensation controls
 - iii. Specimen (samples) - Select the wells to acquire the samples
- 4) In the Plate window, adjust the Loader Settings for the wells.
Ensure each well contains sufficient volume + dead volume
- 5) Right click in the Acquisition Dashboard and choose “Show Plate Control”.
- 6) Make sure that you are set to record the right number of events from the desired gate.
- 7) **Make sure the cytometer is on RUN mode**
- 8) Select the set-up control wells in the plate layout and click “RUN Well(s)”.
No data file is saved.
- 9) Adjust the settings to optimize FSC, SSC, threshold and PMTs voltage.
- 10) Select compensation controls and click “Run wells”.
- 11) Select the Specimen well and click “RUN Plate”, or select the wells you want to run and click “RUN Well(s)”

12) At the end of the acquisition a box will appear that run is finished

Running the Daily Clean and Shutting Down

1) Fill the wells of a 96-well plate according to the following table.

Wells	Solution	Volume (µl)
A1-A4	10% bleach	200
B1-B4	DI water	200

2) Remove the safety cover and place the plate on the plate holder.

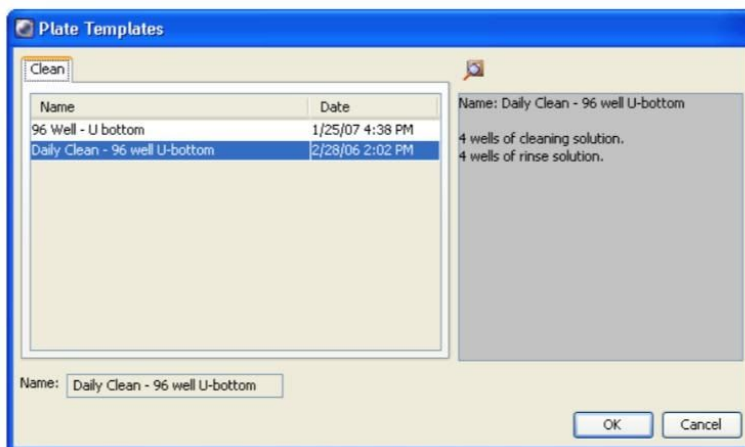
a) Make sure the plate corresponds to the type selected in the software. Orient the plate with well A1 on the back left-corner of the stage. Verify that the sample coupler is properly installed and is not leaking.

3) Replace the safety cover.

4) Place the cytometer in **RUN** mode.

5) Select HTS > Clean.

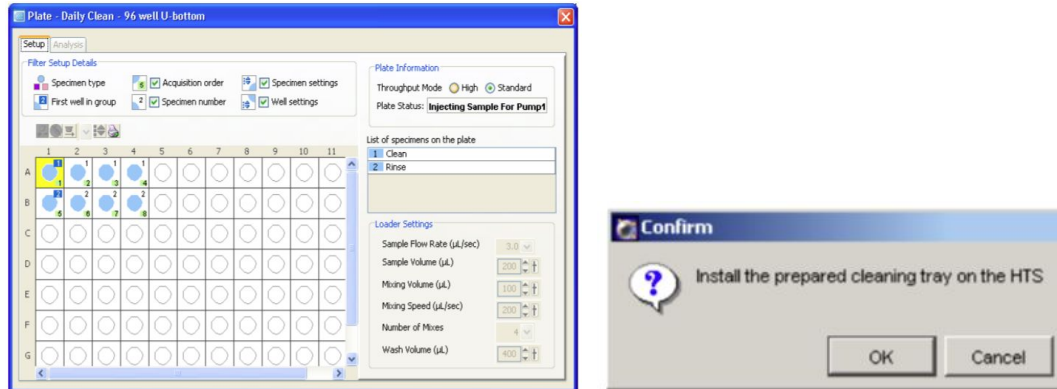
6) The Plate Templates dialog opens.




7) Select the Daily Clean 96-well U-bottom template, if not already selected.

8) Click OK.

- 9) The plate interface changes to show the Daily Clean Protocol view and the Confirm dialog opens.



- 10) Select well A1 (the first well for the cleaning protocol).
- 11) Click OK on the Confirm dialog.
- 12) Click OK when the completion dialog opens.
- 13) Remove and rinse the multiwall plate.
- 14) Make sure the cytometer is still in **RUN** mode. Select **HTS > Prime**.
- 15) When priming is complete, click **OK** in the dialog.
- 16) Place the cytometer in **STANDBY** mode.
- 17) Switch off the HTS power.
- 18) Detach the sample coupler from the cytometer SIT by unscrewing the top thumbscrew. Once the coupler feels free, **GENTLY pull it straight down** from the SIT. **DO NOT pull the coupler at an angle**.
- 19) Remove the SIT protector: Unscrew the tube retainer and slide the SIT protector straight down. **DO NOT pull the SIT protector at and angle**.
- 20) Reinstall the standard droplet containment module (DCM) sleeve: Slide the sleeve straight up over the SIT. Screw the tube retainer on to secure it.
- 21) Install a tube of DI water on the SIT and place the tube support arm under the tube.
- 22) Switch the acquisition control switch to tube mode .
- 23) Shut down the Fortessa.

