

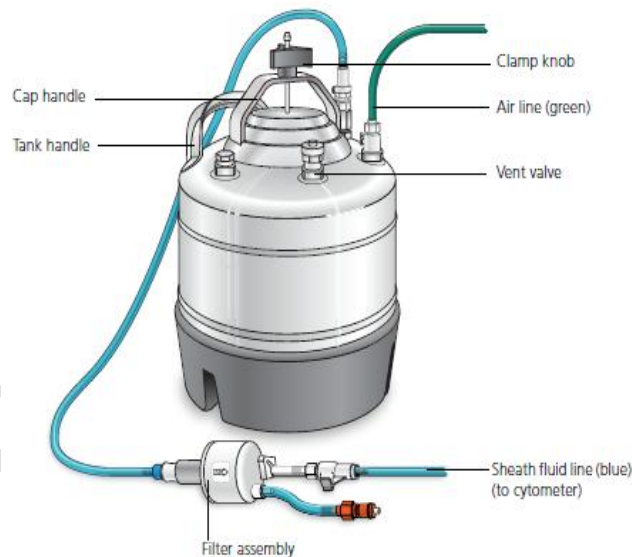
FACS LSRFortessa Standard Operation Protocol HTS Mode


You are strongly recommended to adjust voltage for each channel and setup compensation with Tube Mode before plate mode acquisition!

(Please refer to LSR Fortessa Standard Operation Protocol – Basic operation for Tube Mode)

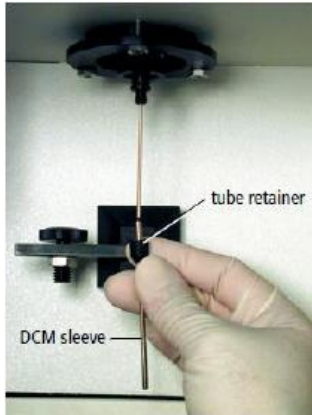
Switching to Plate Mode (HTS Mode):

- Verify that the flow cytometer is in **Standby** mode. Press the **STANDBY** button on the control panel if necessary.
- Click “Cytometer” > “Standby” in Diva Software.
- Disconnect the air line (**Green**) and fluid line (**Blue**) from “**Tube Mode**” sheath container.



- Check the sheath fluid level in “**HTS Mode**” sheath container. Please refill sheath container with sheath fluid for “**HTS Mode**” if necessary.
- Make sure the lid of sheath tank is tightened.
- Reconnect the air line (**Green**) and fluid line (**Blue**) to “**HTS Mode**” sheath container.
- Switch the acquisition control switch to plate mode ().
- Remove the tube of DI water from the SIT.
- Reconnect Diva software with workstation by “Cytometer” > “Connect”.

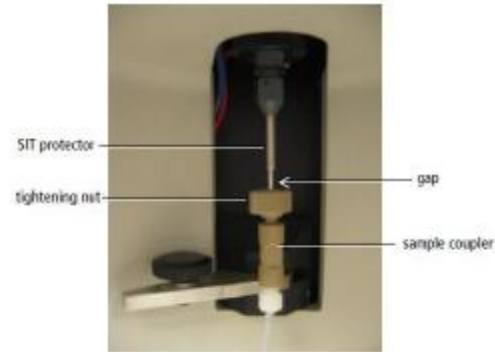
- Remove the DCM sleeve: please unscrew the tube retainer that holds the DCM sleeve onto the SIT and carefully remove the sleeve.
- Install the SIT protector: please slide the protector over the SIT, and push up on the tube retainer until you can screw it onto the SIT. Tighten the tube retainer.
- Attach the HTS sample coupler to the cytometer SIT. Slide the sample coupler onto the SIT until you reach a hard stop. Make sure the sample coupler tubing is not kinked or twisted.



Remove the DCM sleeve



Install the SIT protector



Attach the HTS coupler

- Turn on the HTS.



- Press the **RUN** button on the control panel
- Choose **HTS> Reinitialize** in Diva.
- The following message appears when re-initialization is complete. Click OK.
- Choose **HTS>Prime X6**.
- HTS Mode is ready to use.

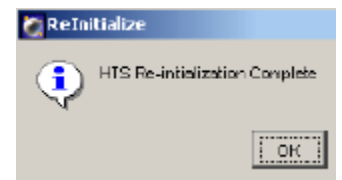
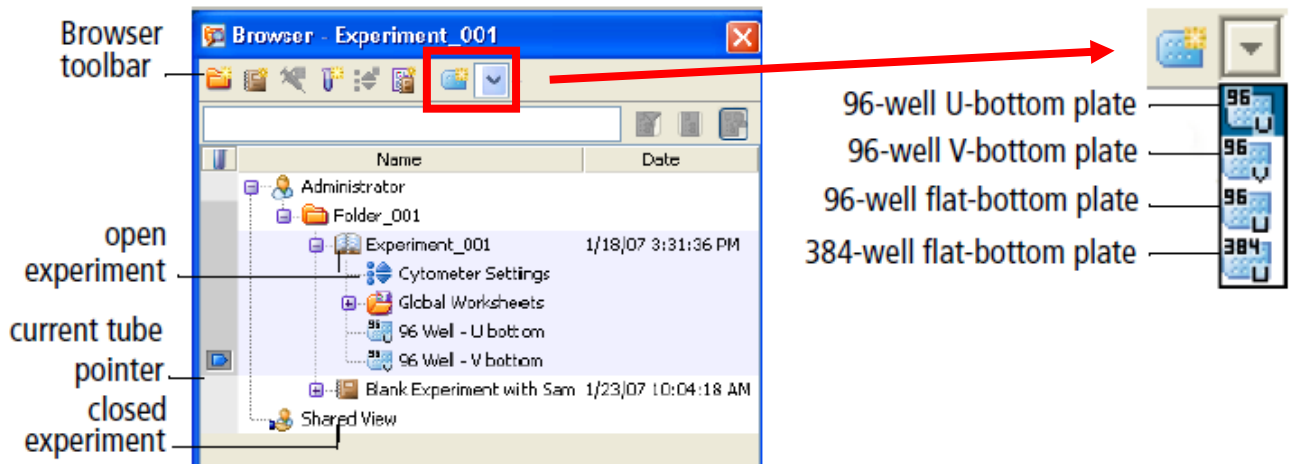
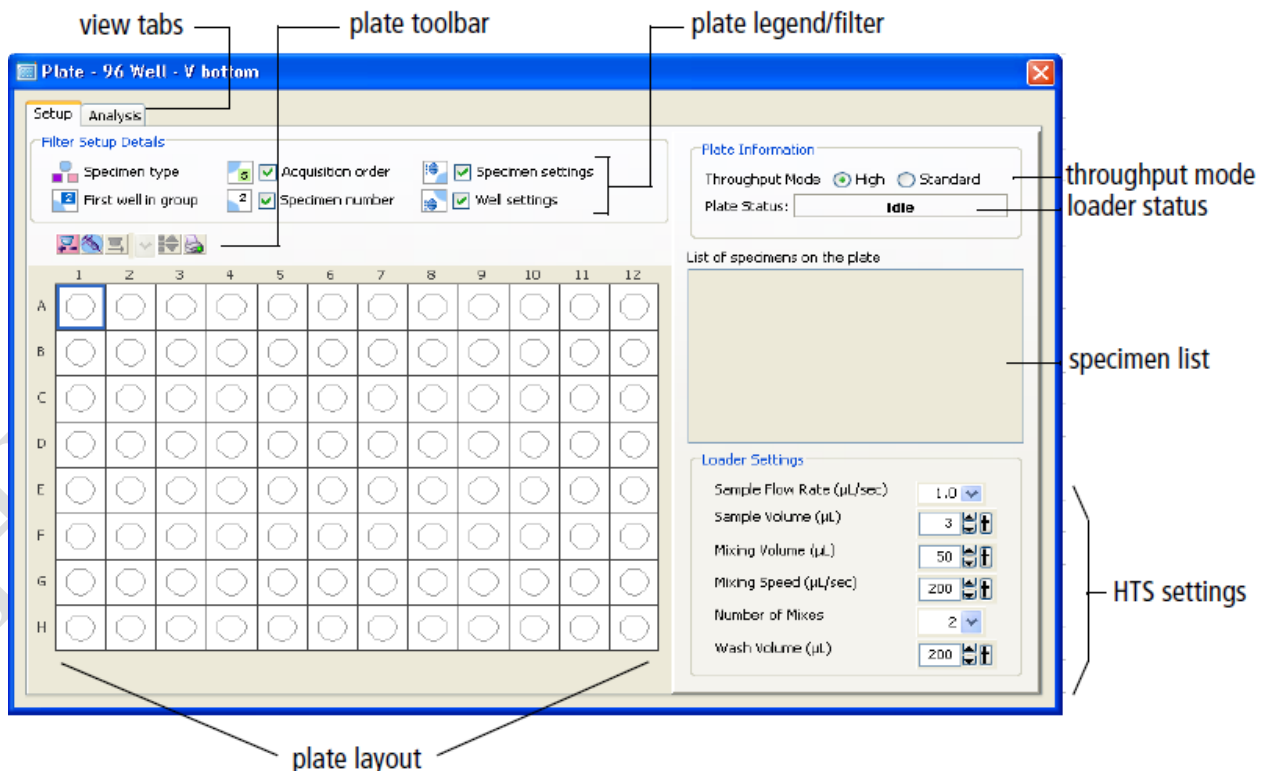


Plate mode acquisition:

- Use the Browser window to create and set up experiments if necessary.
- Click the **New Plate button** in the Browser toolbar to add a default 96-well U-bottom plate to the open experiment. Click the arrow next to the New Plate button to choose a new plate type to add to the experiment.





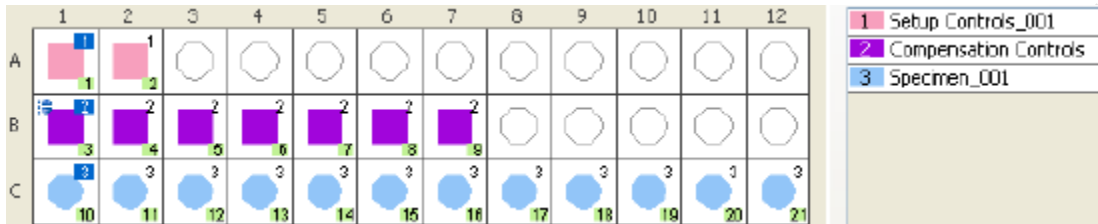
- Plate Window is shown as below:



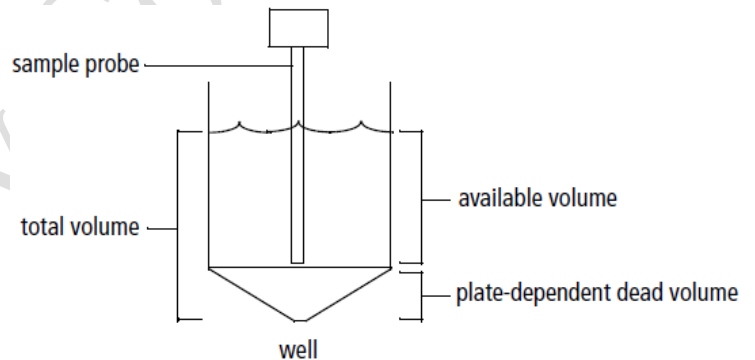
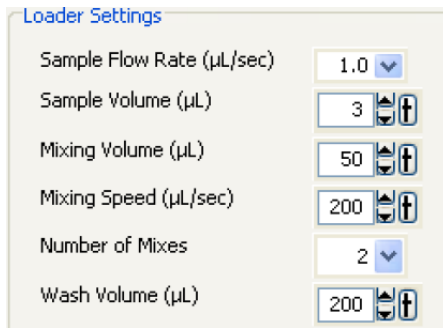
- Set up plate-based experiments in the Plate window.



- Select a well or group of wells, and then click the **Add Setup Controls** button .
- Select a well or group of wells, and then click the **Add Specimen Wells** button .



- The loader (HTS) settings can be modified for the selected well or plate. Default loader settings are provided for each throughput mode. You will need to optimize these settings for the plate type and assay you are running.



Available volume = Volume pipitted into well – aspirated excess volume (20ul) – dead volume

Make sure each well on your plate contains sufficient sample for mixing. Insufficient volume can introduce air bubbles into the system. BD recommends a mixing volume that is one-half the available volume.

- **Minimum sample volume:**
 - 250ul/well for a 96 well plate in standard mode
 - 100ul/well for a 96 well plate in high throughput mode
 - 50ul/well for a 384 well plate (both modes)

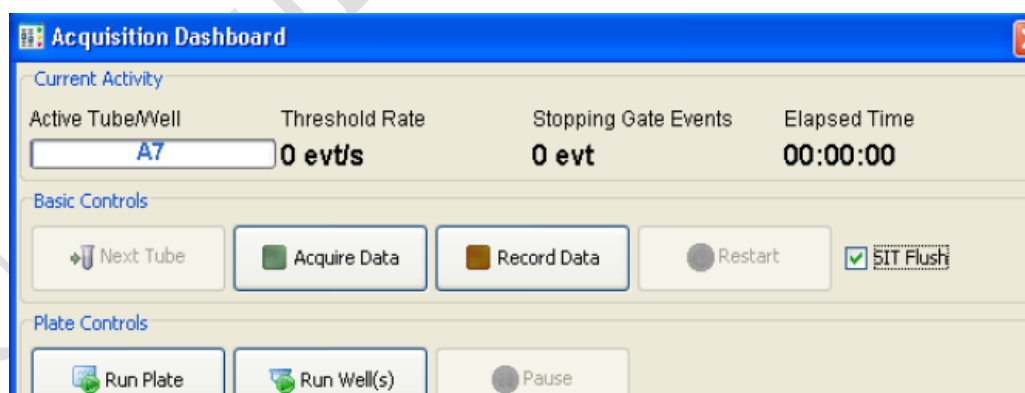
Table 2-4 HTS settings for standard and high throughput mode

Setting	Standard Mode		High Throughput Mode	
	Default	Range	Default	Range
Sample Flow Rate (µL/sec)	1	0.5–3.0	1	0.5–3.0
Sample Volume (µL)	10	2–200	3	2–10
Mixing Volume (µL) ^a	100	5–100	50	5–100
Mixing Speed (µL/sec)	180	25–250	200	25–250
Number of Mixes (cycles)	2	0–5	2	0–5
Wash Volume (µL)	400	200–800	200	200–800

- Select throughput mode by clicking the corresponding mode button in the Setup view.



- Use the HTS controls to acquire and record wells in. Use the Acquisition Dashboard to acquire and record well data. Run Plate runs the wells from the current position to the end of the plate. Run Well(s) runs the selected wells only.



Export Data

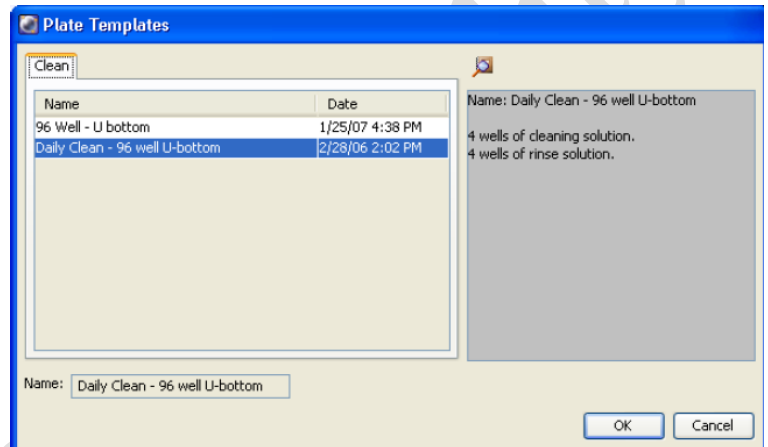
Please refer to “**FACS LSRFortessa Standard Operation Protocol- Basic Operation**”.

Cleaning the System

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

Wells	Solution	Volume (ul)
A1-A4	FACS Clean	200ul
B1-B4	FACS Rinse	200ul
C1-C4	DI Water	200ul

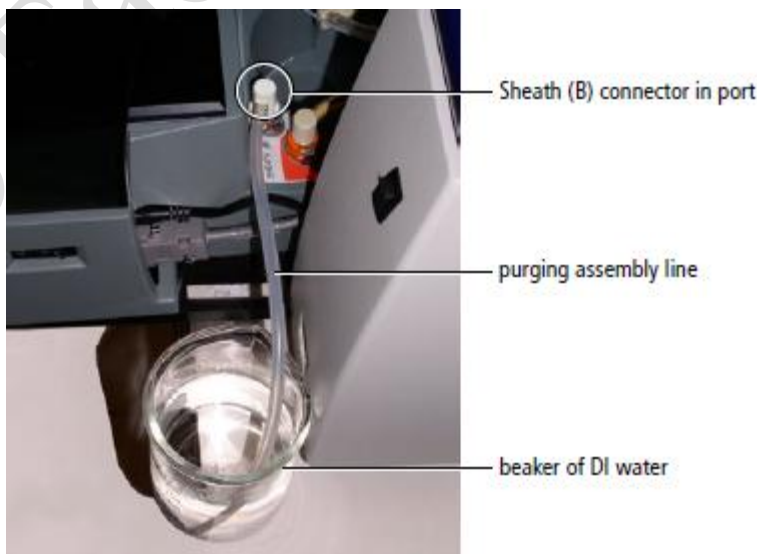
- Place the plate on the plate holder.
- Place the cytometer in Run mode.
- Choose HTS > Clean.
- The Plate Templates dialog appears.



- Select the Daily Clean - 96 well U-bottom template. Click OK.
- The following message appears. Click OK.

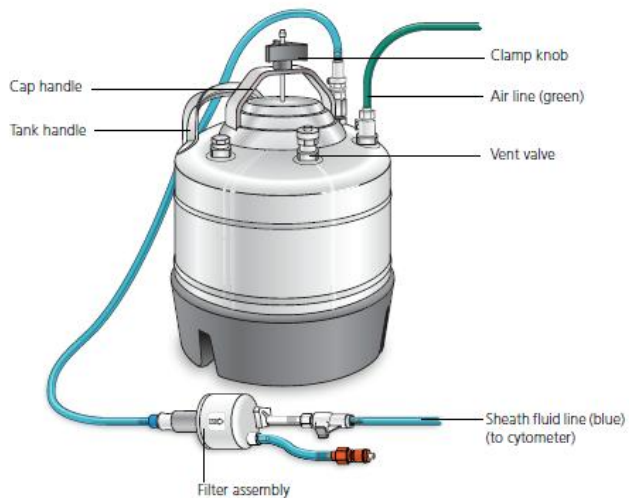


- Click OK when the completion message appears.
- Remove the multiwell plate; rinse it for use on another day.
- Prime the HTS 6 times with DI Water.



Returning to Tube-Mode Acquisition

- Verify that the flow cytometer is in **Standby** mode. Press the **STANDBY** button on the control panel if necessary.
- Click “**Cytometer**” > “**Standby**” in Diva Software.
- Disconnect the air line (**Green**) and fluid line (**Blue**) from “**HTS Mode**” sheath container.



- Check the sheath fluid level in “**Tube Mode**” sheath container. Please refill sheath container with sheath fluid for “**Tube Mode**” if necessary.
- Make sure the lid of sheath tank is tightened.
- Reconnect the air line (**Green**) and fluid line (**Blue**) to “**Tube Mode**” sheath container.
- Switch off the HTS power.
- 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. To avoid bending the SIT, pull straight down on the coupler. Do not pull the coupler at an angle.
- Remove the SIT protector. Unscrew the tube retainer and slide the SIT protector straight down. To avoid bending the SIT, do not slide the SIT protector at an angle.
- Reinstall the standard DCM sleeve. Slide the sleeve straight up over the SIT, and screw the tube retainer on to secure it.
- Install a tube of DI water on the SIT, and place the tube support arm under the tube.
- Switch the acquisition control switch to **tube mode**.
- Reconnect Diva software with workstation by “**Cytometer**” > “**Connect**”.
- **Prime X3.**
- HTS Mode is ready to use.