LSRFortessa Operation Protocol – HTS Loader

Make sure the following actions have been taken before running your samples.

- ❖ Make sure that the waste tank is empty. If not get a prepared waste container from the closet in the hallway
- ❖ Make sure that the bottle with DI water and the FACS Flow container is fully filled

1. **turn on the computer**
   - ❖ Login into Windows using User Name **BDAdmin** and password: **BDIS#1$$**.
2. **Turn on the FACS flow supply system**
3. **Turn on the main power switch of the cytometer** (Green button right side)

❖ **Please make sure that the green lights on both - instrument BD LSR-Fortessa and FACS Flow system are actually "ON".**

Wait 30 minutes for the lasers to warm them up before run your samples

4. **Log into PPMS using your User Name and Password**
5. **Launching the BD FACS DIVA Software**

❖ Log in FACS DIVA software with your personal login name and password.

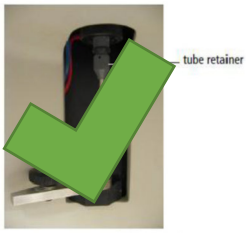
❖ **Always** click **"Use CST Settings"** when pop-up message as below appears.

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

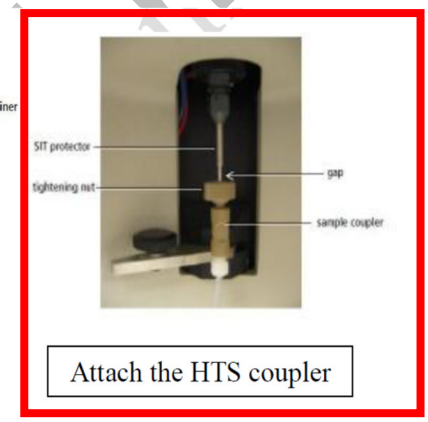
6. Switching to Plate Mode (HTS Mode):

- 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
- Switch the acquisition control switch to plate mode ( )
- Remove the tube of DI water from the SIT
- 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



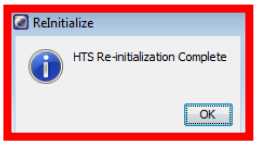
Install the SIT protector



Attach the HTS coupler

- Reconnect Diva software with workstation by "Cytometer" > "Connect"

- Turn on the HTS loader
- Press the **RUN** button on the control panel
- Choose **HTS> Reinitialize** in Diva

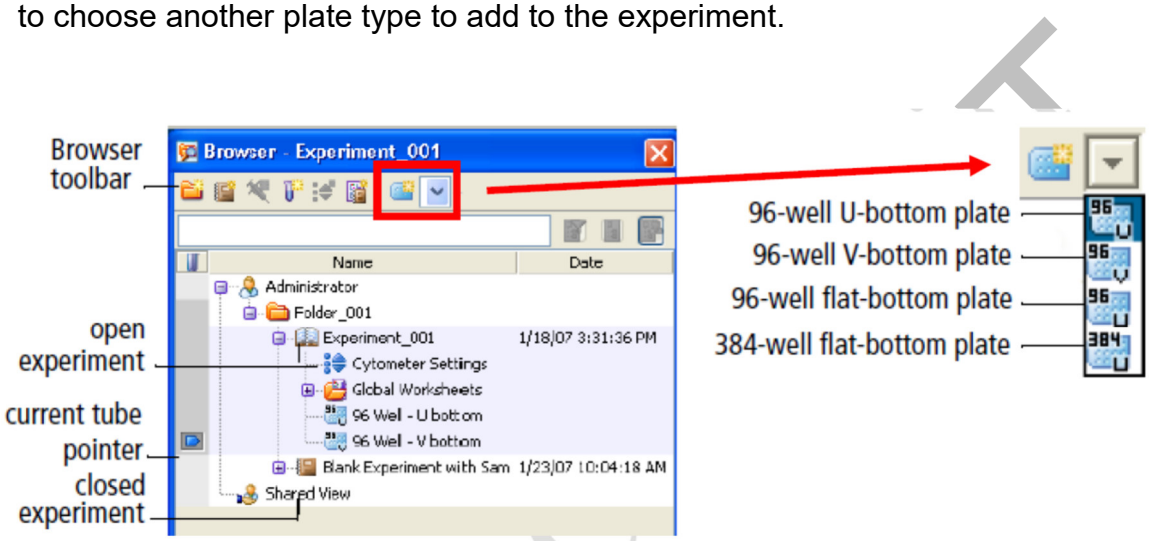


- The following message appears when re-initialization is complete. Click OK.

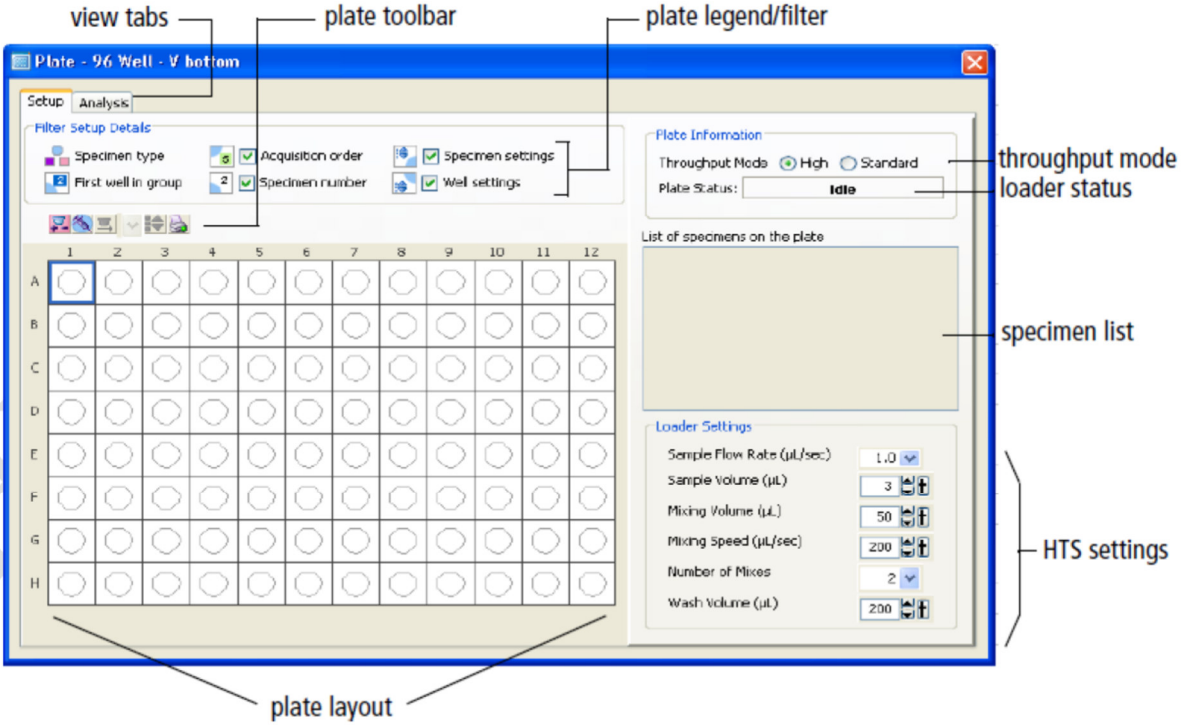
- Choose **HTS>Prime** 3x
- HTS Mode is ready to use

7. 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 another plate type to add to the experiment.



- Plate Window is shown as below:

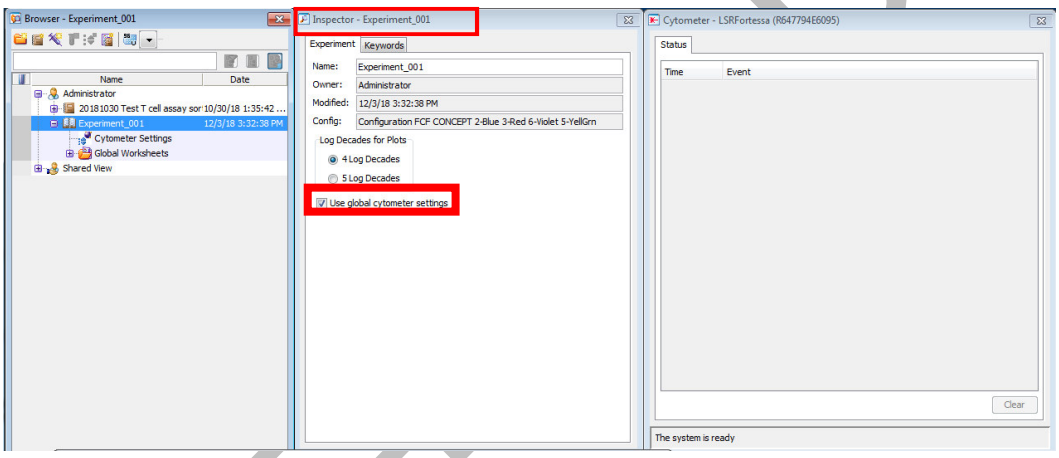


- Set up plate-based experiments in the Plate window

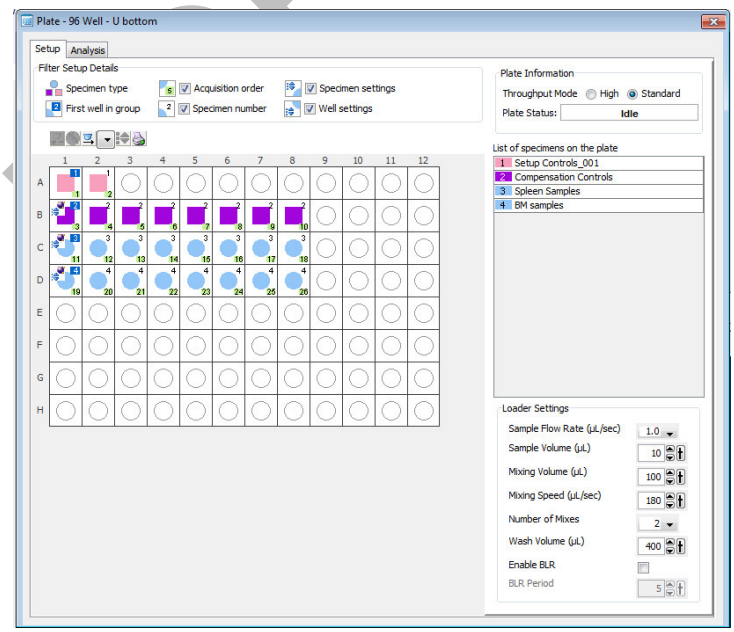
- *Select a well or group of wells, and then click the **Add Setup Controls** button* 
  (only necessary if compensation has not already been performed in tubes)

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

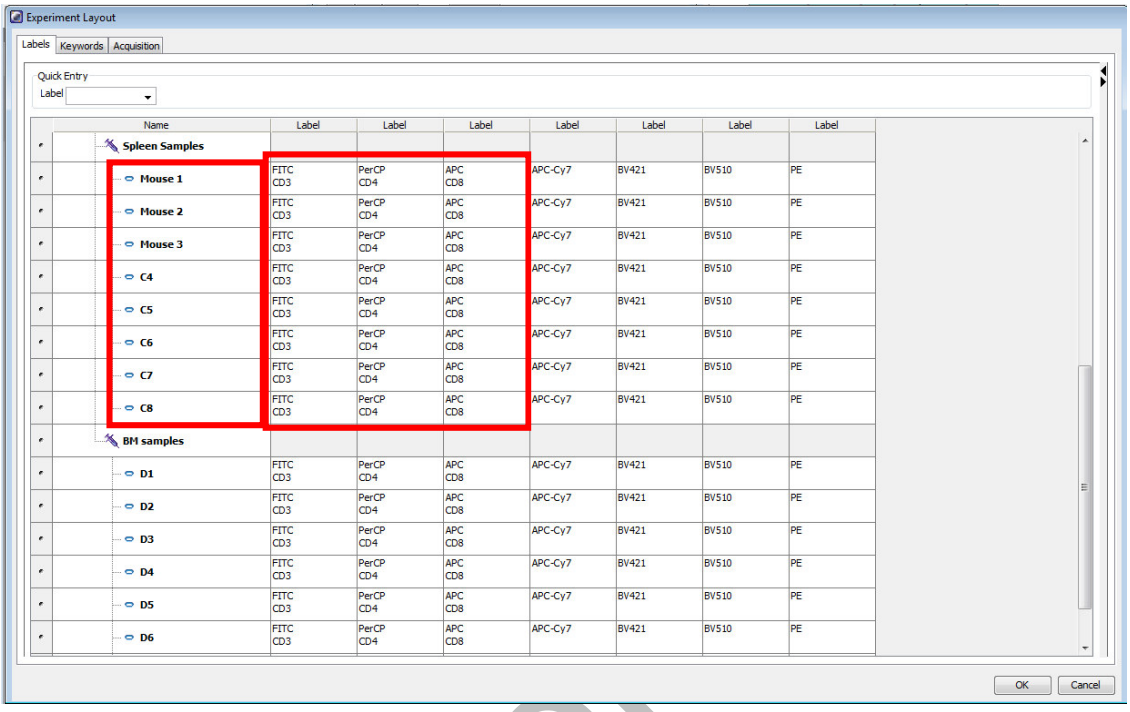
- Assign cytometer settings by click the **Add Cytometer Settings** button 
  The system will automatically use Global Cytometer Settings
  If you want to **assign different cytometer settings at one plate, remove the checkmark "Use Global Cytometer Settings" in the Inspector** → Experiment panel
  Select a group of wells → right click → Setup → link Setup → select the preferred setup out of the list



Example for a 96 U-well plate

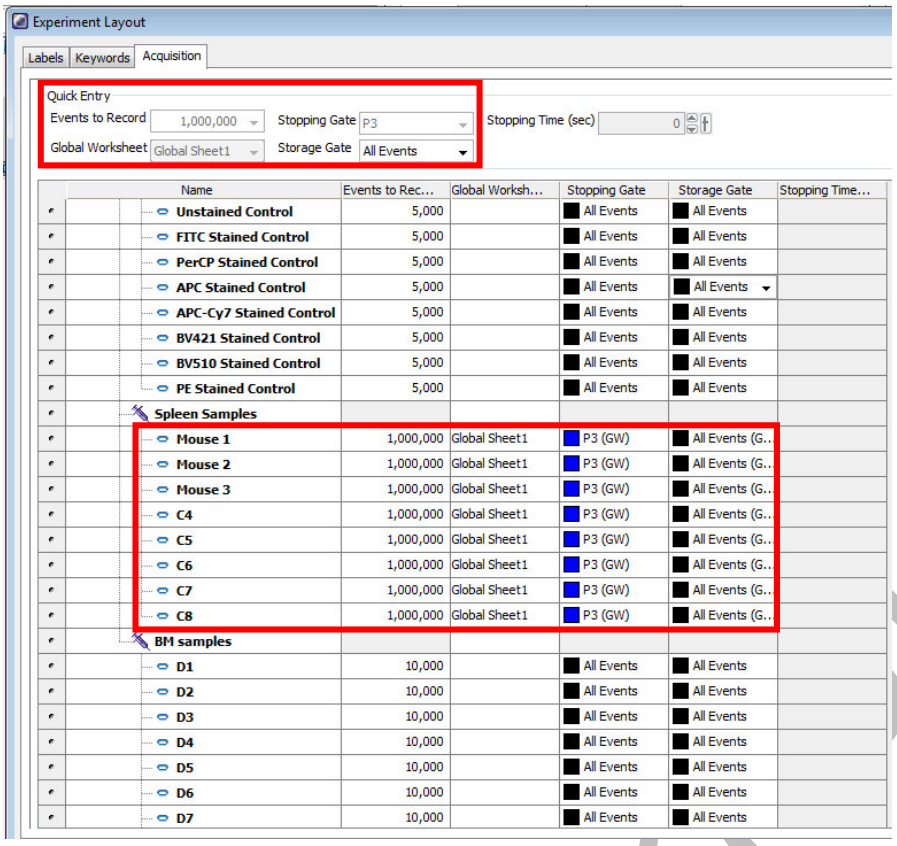


- Name your samples and selected fluorophores via **Experiment → Experiment Layout**



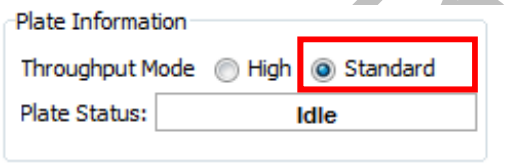
- Set the number of events to record via **Experiment → Experiment Layout → Acquisition**

- Set the right Gate for the Stopping Gate via Experiment → **Experiment Layout → Acquisition Stopping Gate**

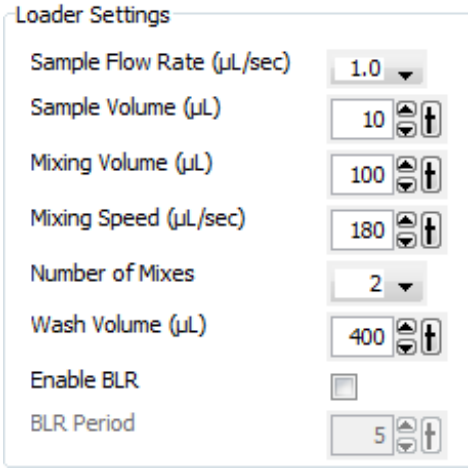


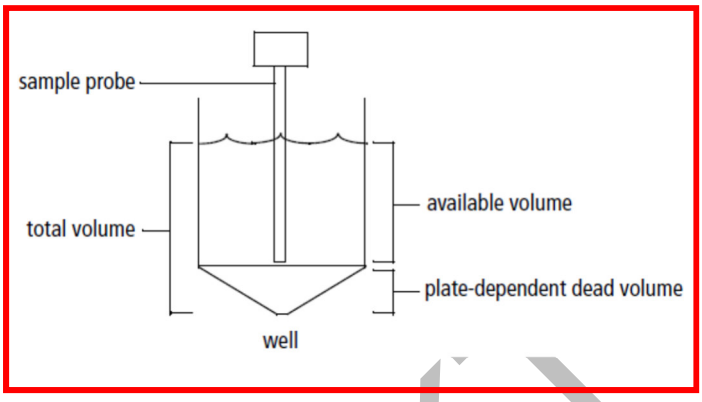
Name	Events to Rec...	Global Worksh...	Stopping Gate	Storage Gate	Stopping Time...
Unstained Control	5,000		All Events	All Events	
FITC Stained Control	5,000		All Events	All Events	
PerCP Stained Control	5,000		All Events	All Events	
APC Stained Control	5,000		All Events	All Events	
APC-Cy7 Stained Control	5,000		All Events	All Events	
BV421 Stained Control	5,000		All Events	All Events	
BV510 Stained Control	5,000		All Events	All Events	
PE Stained Control	5,000		All Events	All Events	
Spleen Samples					
Mouse 1	1,000,000	Global Sheet1	P3 (GW)	All Events (G...	
Mouse 2	1,000,000	Global Sheet1	P3 (GW)	All Events (G...	
Mouse 3	1,000,000	Global Sheet1	P3 (GW)	All Events (G...	
C4	1,000,000	Global Sheet1	P3 (GW)	All Events (G...	
C5	1,000,000	Global Sheet1	P3 (GW)	All Events (G...	
C6	1,000,000	Global Sheet1	P3 (GW)	All Events (G...	
C7	1,000,000	Global Sheet1	P3 (GW)	All Events (G...	
C8	1,000,000	Global Sheet1	P3 (GW)	All Events (G...	
BM samples					
D1	10,000		All Events	All Events	
D2	10,000		All Events	All Events	
D3	10,000		All Events	All Events	
D4	10,000		All Events	All Events	
D5	10,000		All Events	All Events	
D6	10,000		All Events	All Events	
D7	10,000		All Events	All Events	

- Select throughput mode by clicking the corresponding mode button in the Setup view (usually standard mode)



- Wells will be acquired in the order they were created (Order: see small number in the lower right corner)
- 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.





The sample probe is for BD plates

Available volume = Volume pipetted 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.

Maximum sample volume:

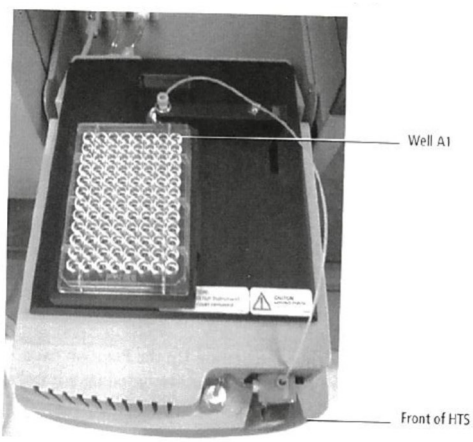
- **250µl/well for a 96 well plate in standard mode**
- **100µl/well for a 96 well plate in high throughput mode**
- **50µl/well for a 384 well plate (both modes)**

The sample needle sucks up the entire volume except the dead volume

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

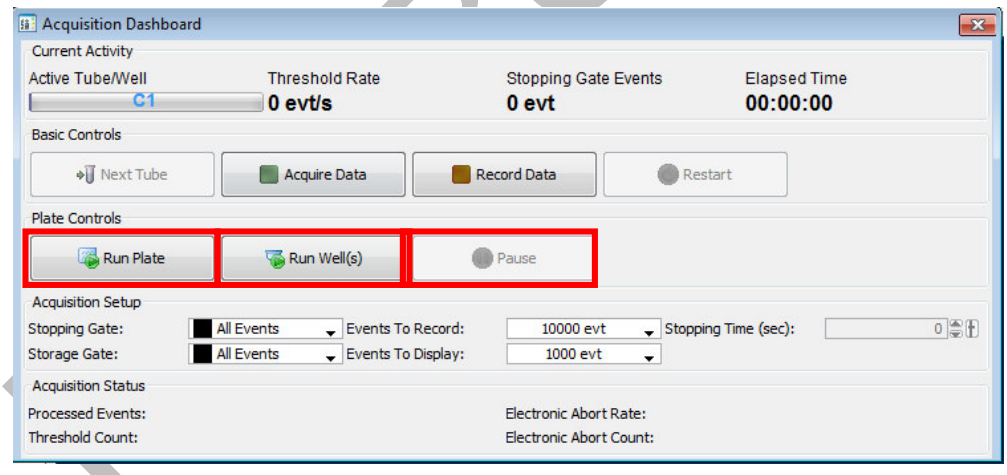
- Remove the safety cover on the HTS unit and place the prepared plate on the plate holder.
  To prevent damage of the HTS probe, always make sure to remove the multiwell plate cover before you put the plate on the holder!!!



- Replace the HTS safety cover.
  Make sure that the sample line is not affected by the cover.
  The HTS is equipped with a safety interlock that prevents the cytometer from running when the safety cover is removed or opened. Do not remove or open the safety cover while samples are being processed.

- Press the RUN button on the cytometer.
  Do not put the LSR Fortessa in Standby during HTS acquisition. Run cannot be continued when cytometer is in standby mode, and it can cause damage to the flow cell.

- 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.



- to pause a run, click **Pause** in the acquisition dashboard
  HTS finishes processing the current well
  to continue: click **Resume**
  to stop the run: click **Stop Well(s)** or **Stop Plate**

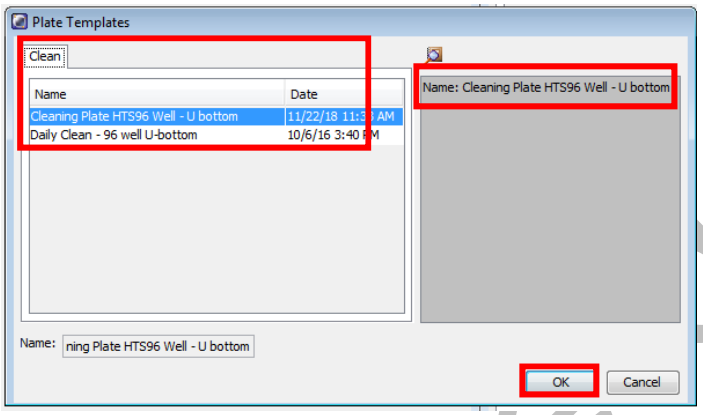
  Note: During the run it is possible to change the FSC/SSC settings in between the sample acquisition

7. Cleaning the system

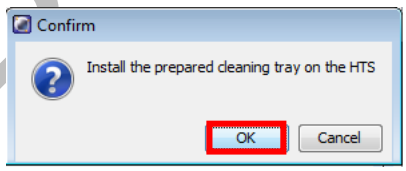
- Fill the wells of a 96-well plate according to the following table.

Wells	Solution	Volume (µl)
A1-A4	DI Water	250 µl
B1-B4	FACS Clean	250 µl
C1-C4	FACS Rinse	250 µl
D1-D4	DI Water	250 µl

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



- Select the Cleaning Plate HTS - 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 3x with DI Water.

8. Return 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
- 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.
- 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** 3x.
- Tube Mode is ready to use

Note: *Please report all the problems/concerns to FCF Berg Staff:*
During business hours *— get to Room 581 and tell the FCF Berg staff member.*
After hours *— e-mail to fcf-berg@med.uni-tuebingen.de; leave the note about the problem on the instrument's keyboard*