General

Registration with the Flow Cytometry Facility, as well as attending the technical introduction by the facility staff is a prerequisite to use the Cytek Aurora**.**

Questions / reporting technical problems:

Feedback form

Emergencies:

044 635 02 03
044 635 02 06

The Plate loader works with:

- **Standard size 96 well (U-, V- or flat-bottom) plates.**

Differences to the loader on the Aurora 2:

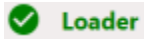
- **No tube rack or deep well plate loading.**
- **Can cool and heat the plate.**
- **Cell suspension via stir bar.**

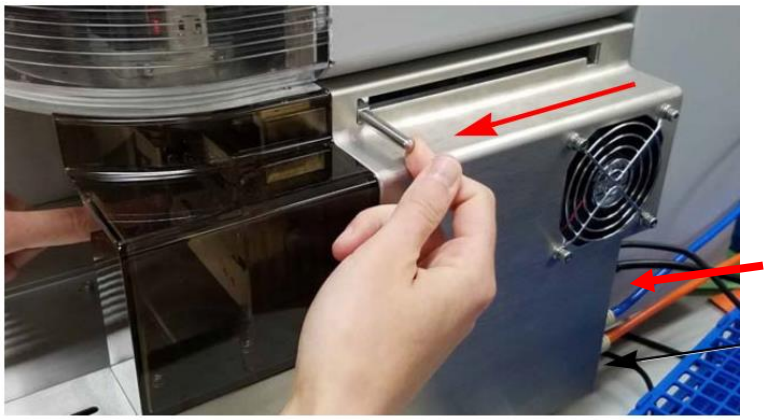
The officially supported plates according to the manufacturer are:

Plate Type	Material	Part Number
96-well flat-bottom	Polypropylene	VWR 29444-100
	Polystyrene	VWR 29442-070
96-well U-bottom	Polypropylene	VWR 29444-104
	Polystyrene	VWR 29442-396
96-well V-bottom	Polypropylene	VWR 29444-102
	Polystyrene	VWR 29442-402

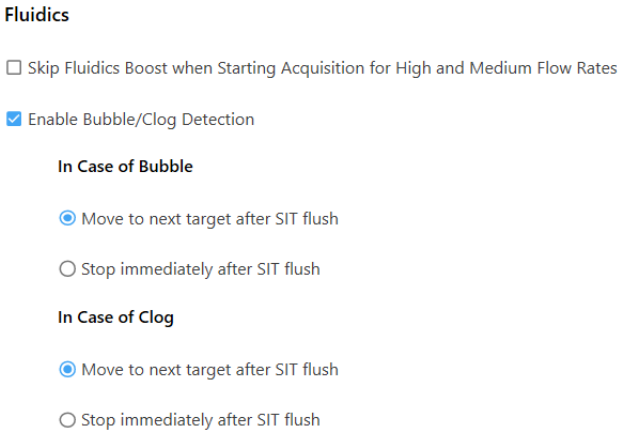
I. Switching from Tube to Plate Acquisition

1. Make sure that there is no tube on the SIP and that the sample line is pulled into the instrument. If a tube is still attached or the sample line is lowered, the system will be damaged by the next step!
2. Pull the lever to the front.
3. Switch on the plate loader via the switch at the back.
4. Wait for the Loader status display in the software to turn green:





5. Go to Preferences → Cytometer and make sure that the “Enable Bubble/Clog Detection” is turned on in the Fluidics section. Choose what should be done in case of bubble or clog detection.
6. If plate tray is not at the front, click the Eject button in the Cytometer tab or the Acquisition Control.
7. Put your plate on the tray. **A1 position is at the front left.**
8. Load the plate (move it to the back of the loader) by clicking Load in the Acquisition Control or the Cytometer tab.



9. Go to the Cytometer tab and perform a Calibrate SIT. Set the Lift Distance to the appropriate value for your plate (see below). If Calibrate SIT grayed out: switch to experiment and back again.
10. The instrument is now ready to acquire the plate.

II. Verify SIT calibration

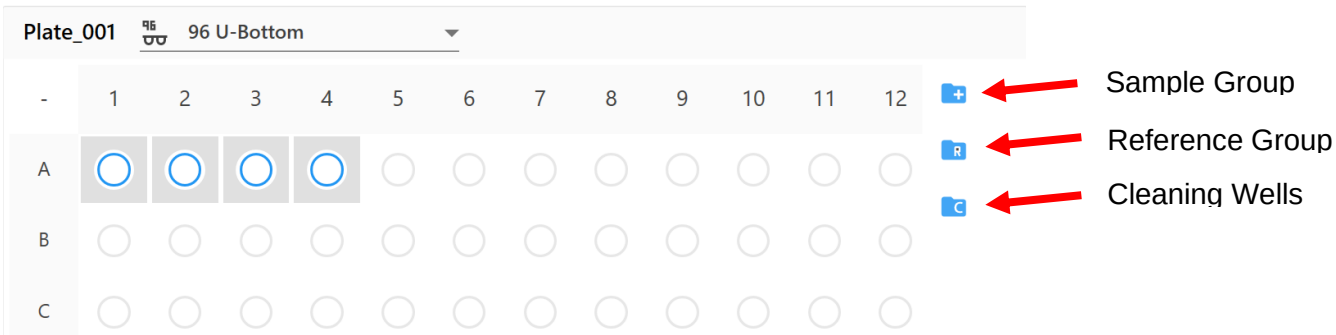
This step is recommended whenever you use a certain type of plate for the first time.

Note: Lift distance settings from other Auroras are not necessarily transferrable between instruments.

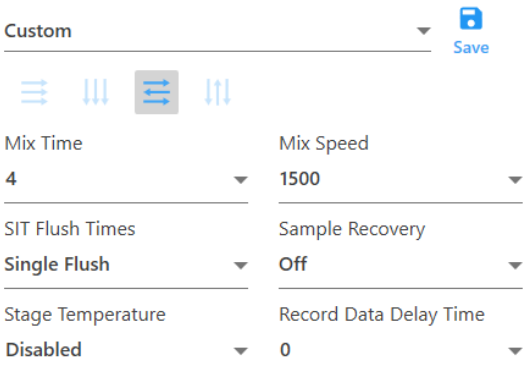
1. Fill a well with ddH_2O and load the plate.
2. Acquire from the well and check the flow rate.
   Expected flow rates in plate mode are:
   - Low: 15 ul / min
   - Medium: 30 ul / min
   - High: 100 ul / min
3. If the measured flow rate is too low or you get a “Clog detected” message, go to the Cytometer tab and perform a Calibrate SIT with a higher Lift Distance. Standard value is 0.75.
4. Repeat steps 2 and 3 until you get the desired sample flow rate.

III. Set up plate in your experiment

1. Create a new experiment or edit an existing one.
2. Change carrier type to the plate type you are using.
3. Under Groups, add the plates you want to acquire.
4. Select the appropriate wells and click the buttons on the right to create groups for normal samples, reference controls or cleaning wells (are processed but not recorded).



5. Set the loader settings. Choose from Default, High Througput or Low Carryover or create your custom settings.



Acquisition order	• row from left to right (A1-A12, B1-B12, etc) • column from top to bottom (1A-1H, 2A-2H, etc) • row from left to right, then right to left (A1-A12, B12-B1, C1-C12, etc) • column from top to bottom, then bottom to top (1A-1H, 2H-2A, etc)
Mix Time	Time in seconds each well gets stirred before acquisition.
Mix Speed	Select the speed at which the mixer spins (in RPM).
SIT Flush Times	Number of SIT flushes performed after each acquisition.
Sample Recovery	Allows any remaining sample that is left in the SIT after acquisition to be deposited back into the well.
Stage Temperature	Stage temperature (4°–30°C) in rough increments.
Record Data Delay Time	Time in seconds before recording starts (to e.g. exclude the boost phase).

IV. Cleaning the Loader

1. Fill 3 wells with 300 ul bleach (FACSClean) and two wells with 300 ul water.
2. Run the wells (wells with bleach first) as cleaning wells with the mixing time set to maximum. Acquire 10 ul per well at high flow rate (6 sec / well).
3. Run the standard cleaning procedure after reverting back to tube mode.

V. Change back to Tube loading

Washing of the instrument can only be done in tube mode. Therefore, every user must revert the instrument back to tube loading at the end of acquisition.

4. Make sure that the sample line is retracted into the instrument. If not, you will break it in step 8.
5. Remove your plate from the loading tray.
6. Click on “Load” in the Acquisition Control or in the Cytometer tab to move the tray to the back.
7. Switch off the plate loader.
8. Move the mixer to the back by pushing the lever to the back.
9. Change the carrier type back to manual tube loading
10. Run the cleaning procedure as described in the Cytek Aurora SOP.