Aurora

Operation Protocol

Loader

FCF-Berg

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Index

Using the Loader
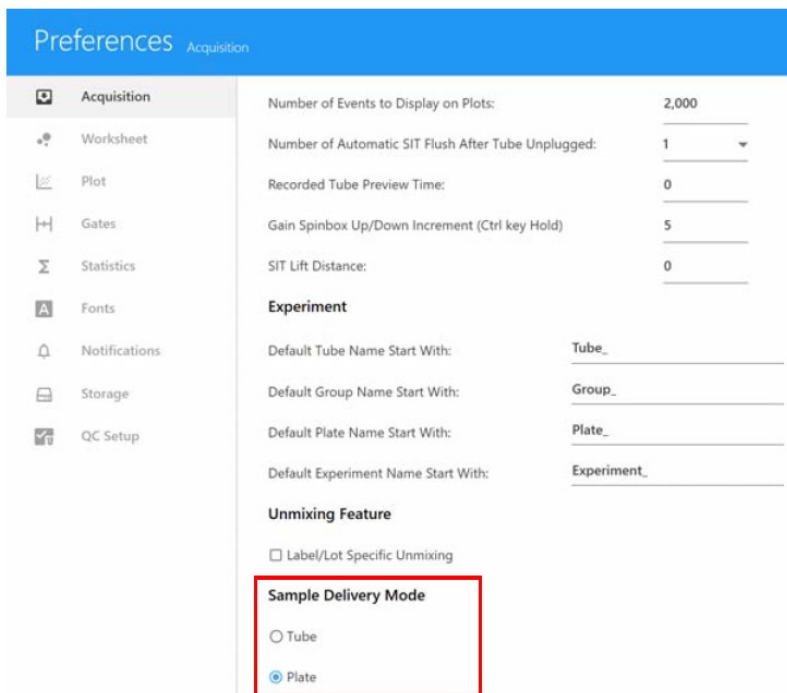
1) Turn on the power to the Loader. The power switch is at the back of the Loader where the fluidic lines connect.

2) If there is a tube on the SIP, remove it.
3) Pull the lever towards you to bring the mixer forward.
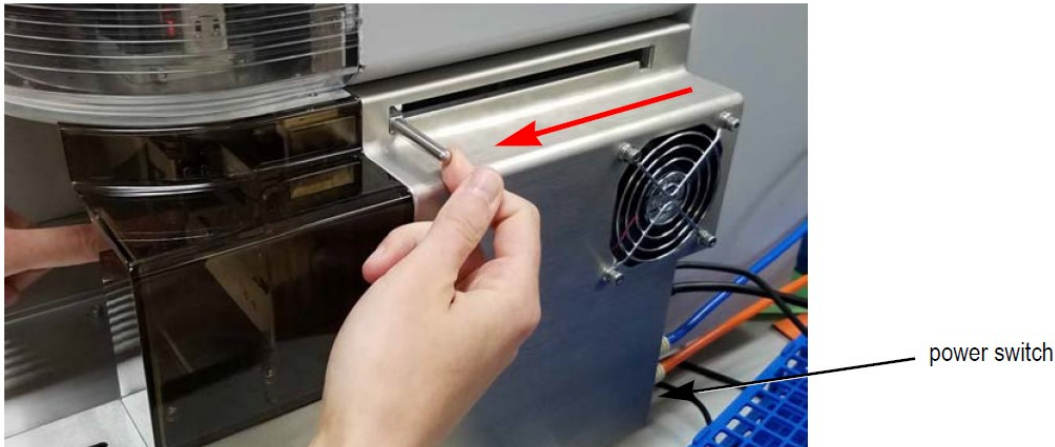
4) Set the **Sample Delivery Mode** in Acquisition **Preferences** to **Plate**.

Minimum Well Volume

When acquiring from a well, a boost is applied to deliver the sample to the flow cell as quickly as possible. This process, as well as mixing, results in the loss (consumption) of up to 75 µL of sample, as the sample is neither acquired nor recorded. We recommend a minimum volume of 150 µL of sample per well, keeping in mind that approximately 75 µL will be lost.

Experiments in Plate Mode

Follow the instructions "*Creating a New Experiment*" in the General Operation Protocol to create a new experiment. The steps to create a new experiment when running in plate mode are the same as when running in Tube mode, except for the following tasks—creating groups and defining Loader settings—outlined below.

Creating Groups When Using the Loader

NOTE: When running sticky samples, such as lyse, no wash (LNW) samples, we recommend adding cleaning wells between samples to thoroughly clean the mixing probe. For example, add two wells, one with 10% bleach and the other with DI water. At the end of a plate, consider adding a group of four wells, two with 300 µL of 10% bleach and two with 300 µL of DI water. Program a long mix (15 seconds at 1500 rpm) to thoroughly clean the mixing probe.

Before you can add groups when creating a new experiment, you must add a plate.

1) To add groups when running in plate mode, click **Add Plate**, then select the **plate type** (96U-,V-, or Flat-Bottom) A plate image appears on the right. Three icons at the far right allow you to add groups.
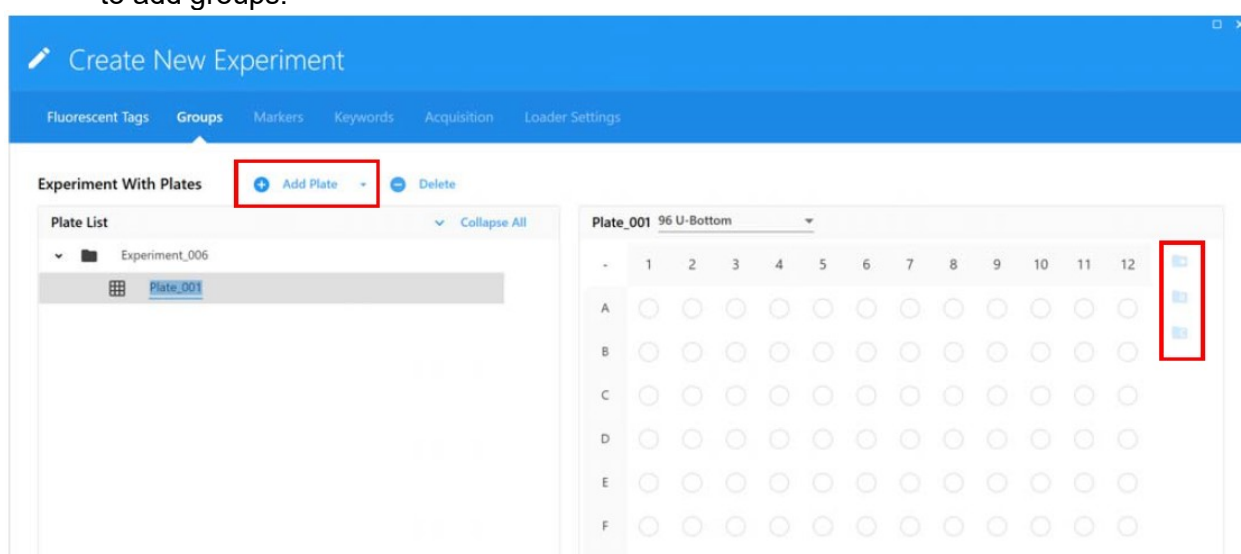
2) Click in the **plate image** to select a well, or click and drag to select multiple wells corresponding to the wells in the group you wish to add, then click the appropriate icon to the right of the plate to define the sample types in the group:

   - To add a group for samples, **click +.**
   - To add a group for reference controls, **click R.**

3) If you are intending to unmix with controls acquired in this experiment you must add a reference group. When you add a reference group you will be prompted to define the reference controls. See *"Creating an Experiment "step 7 in General Operation Protocol* for details on defining the unstained and fluorescence controls.

   - To add a group for cleaning well(s), click **C.** For example, you can add DI water to wells to rinse the SIT and clean the mixing probe to prevent carryover.

   Wells will be marked as sample (S), reference control (R), or cleaning (C).
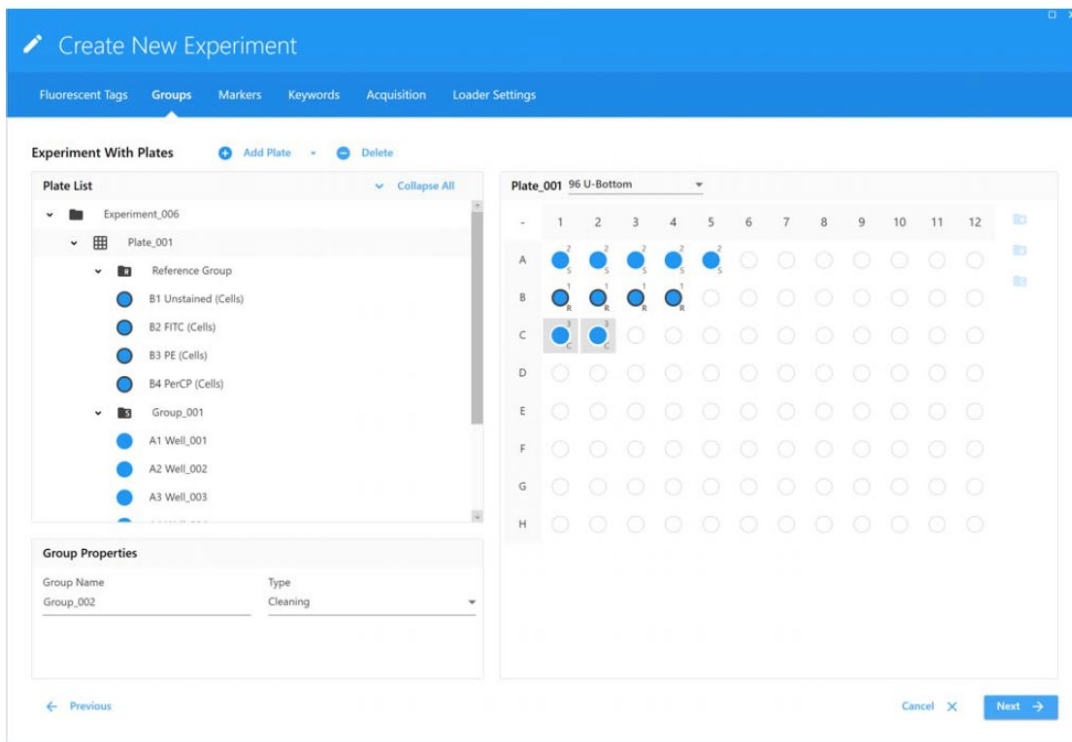
4) When all groups have been defined > click **Next.**

5) Follow steps **13 through 16** of *"Creating an Experiment " in General Operation Protocol* starting to continue creating the experiment.

## Loader Settings in the Experiment

This section lists the differences in the Acquisition module when using a Loader. The following panes are described:

• Group Hierarchy
• Acquisition Control
• Loader Settings

Group Hierarchy/Plate Display

The Group and Tube hierarchy pane when using a Loader defaults to one group with one tube. It also provides an option to add a plate. You can view either a hierarchical list (List View), or once a plate is added, a graphical representation of the plate (Plate View).
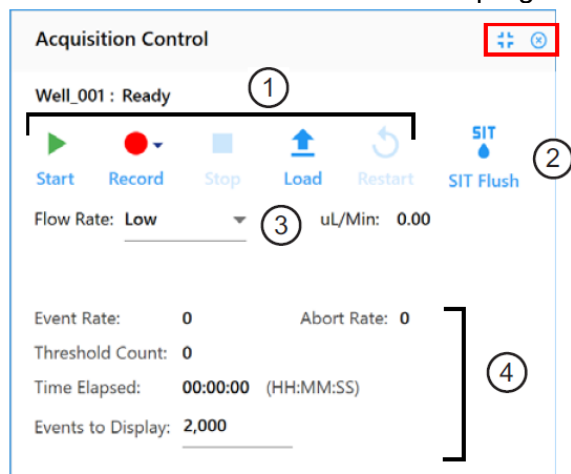
Default Experiment List View
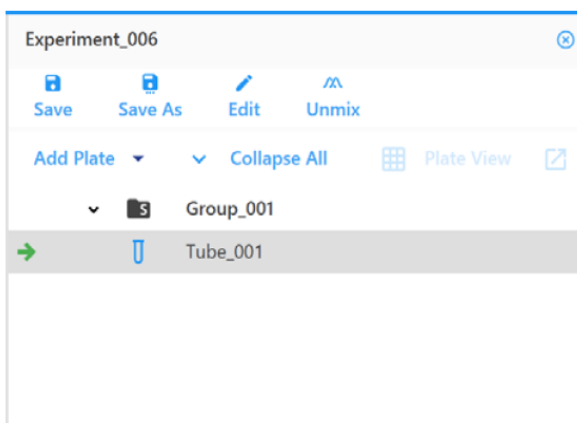
Default Experiment Plate View Once Plate is Added
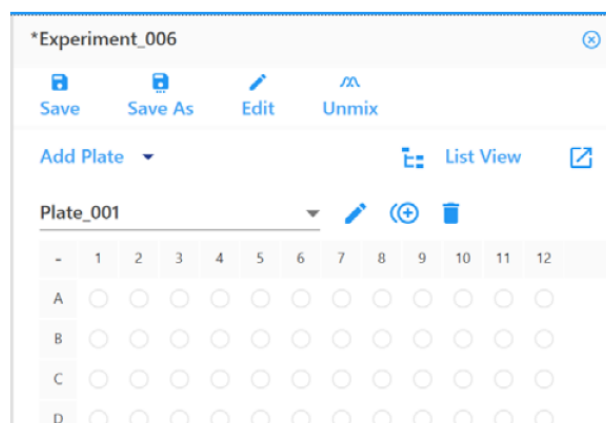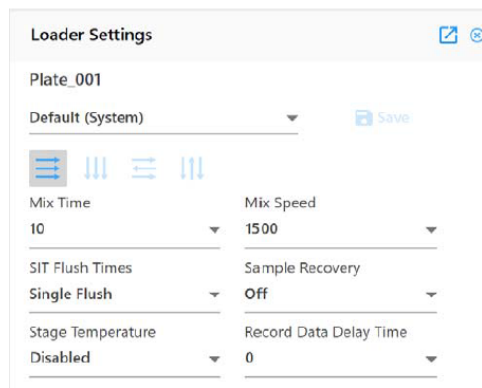
Loader Acquisition Controls

The Acquisition Control pane allows you to start, stop, and pause acquisition, record data, and restart acquisition counters. To show, hide, or undock (float) this pane from the experiment panel, use the dock/undock and hide icons in the top-right corner.

No.	Control	Description
1	Start/Record/Stop/ Load/Eject/Restart	Start and Record are enabled when the Loader is on, the lever is pulled forward, and the Sample Delivery Mode in Acquisition preferences is set to Plate. Select Start to load the plate and start acquisition Select Record to record data. Record can also start acquisition. Select Stop to stop acquisition. Select Load or Eject to load or eject the plate. Select Restart to restart the acquisition counters. All events and results displayed are refreshed. Stop and Restart are enabled once Start is selected. Stop and Pause are enabled once Record is selected.
2	SIT Flush	Select to perform a SIT Flush
3	Flow Rate	Select Low (15 µL/min), Medium (30 µL/min), or High (100 µL/min). The exact flow rate is displayed.
4	Event Rate, Abort Rate, Threshold Count, Time Elapsed	Displays the real-time counts during acquisition.
5	Events to Display	Enter the number of events to display during acquisition.

Loader Settings

Several default Loader settings are available. You can select the Default, High Throughput, or Low Carryover Loader Setting, depending on your application. You can also create custom settings. Loader settings are enabled once a plate is added. Adjust the settings before acquisition.

The following table describes the Loader settings.

Setting	Description
Selected Settings	Three Loader settings are available—default, high throughput, and low carryover. You can create your own custom settings.
Acquisition order	Select the order that you want the plate to run. Wells are acquired by: • 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	Select the time (in seconds) each well gets mixed before acquisition. You can also disable the mix time.
Mix Speed	Select the speed at which the mixer spins (in RPM).
SIT Flush Times	SIT flush is performed over the wash station after each acquisition. Choose single flush, double flush, or disabled if you do not wish to perform a SIT flush.
Sample Recovery	Allows any remaining sample that is left in the SIT after acquisition is complete to be deposited back into the well.
Stage Temperature	Select the plate stage temperature (4°–30°C).
Record Data Delay Time	Select the time in seconds you wish to preview data from a well before recording begins once you click Record.

Calibrating a Plate

The Plate Calibration option is available only to administrators. **If necessary please contact FCF-Berg.**
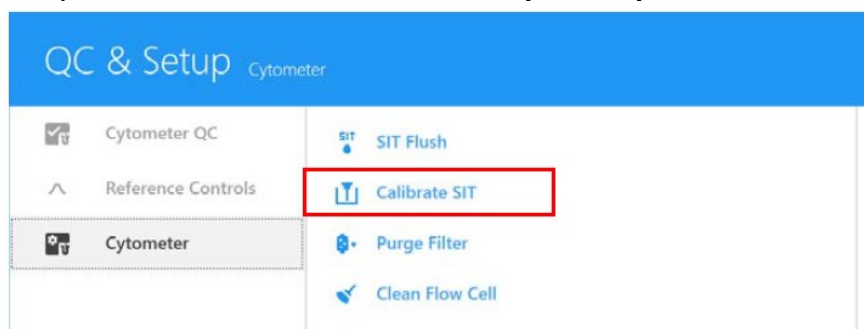
Calibrating the SIT

Use the Calibrate SIT feature to set the proper SIT depth within a plate well. You will need to perform a SIT calibration:

• prior to running your first experiment of the day in plate mode
• every time you switch from tube mode to plate mode
• if you change plate types

Once calibration is complete, the settings will apply to all plates of the same type.

NOTE: A SIT calibration is performed automatically in the tube that is loaded on the SIP when the system is turned on.

1) Load a 96-well plate. (see *Loading a 96 well plate*)

2) Select from either the **QC & Setup or Acquisition module** > **Calibrate SIT.**

3) A dialog appears prompting you to enter the SIT Lift Distance. Enter the lift distance, then click **Calibrate SIT**.

<u>The lift distance is the distance in millimeters from the tip of the SIT to bottom of the well.</u>

4) Check the depth of the sample line in the well. If it's satisfactory, click **OK**. If it needs to be raised or lowered, adjust the SIT Lift Distance and click Calibrate SIT.

5) Repeat step 4 until the sample line depth is satisfactory. Click **OK**.

6) Once SIT calibration is complete, you can run your experiment.

Loading a 96 well plate

1) If necessary, press **Eject** from either the **QC & Setup** or **Acquisition module** to eject the stage, bringing it forward.

2) Load a plate on the plate stage so that **position A1** is located in the front-left corner. Insert the edge of the plate behind the metal tabs at the front of the plate stage, then press down on the back edge of the plate to secure it in the holder.
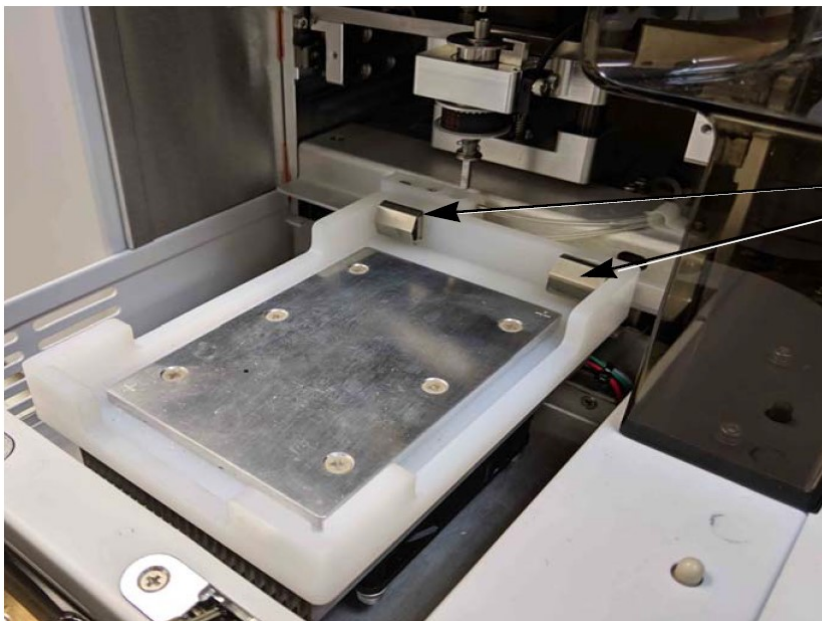
Insert back edge of plate in front of metal tabs, then press down on front edge of plate to secure.

3) Press **Load** from the **QC & Setup** or **Acquisition module** to load the plate, followed by **Start** to begin acquisition.
    Or press **Start** to load the plate and begin acquisition.

Shut Down

After your plate experiment: To switch back to **tube mode**, set the **Sample Delivery Mode** in the Acquisition Preferences back to **Tube** and slide the Loader lever back.

See *Shut Down in General Operation Protocol* for following informations.