FACSLyric™ – General User instructions

In BD FACSuite software, you can measure and analyze samples using either assays or experiments. Use experiments to acquire and visualize samples manually, to develop your assay and to adjust settings. Use assays when you want to run an analysis repeatedly. Assays are run as entries in a worklist, which provides batch acquisition and analysis.

BD FACSLyric™ Startup Procedure

☑ Check whether sheath tank full and waste tank empty. If you empty the waste tank, add 1 l FACS Clean to the tank.

☑ Turn on the power to the system by pressing the Power button. The power button turns green when system power is on.

☑ Turn on the computer, login to windows as Operator [PW Welcome#1].

☑ Start BD FACSuite software. Enter your personal account name and password to log in, and then click OK.

☑ Check the connection and fluidic status in the status bar.

☑ Verify that the 20-minutes laser warmup has been completed. A count-down timer in the status bar will indicate the remaining time.

☑ Perform a SIT-Flush. Select Cytometer > Fluidics > SIT Flush.

☑ Perform daily clean: Place a tube with 2 ml of FACS Clean and a tube containing 3 ml of DI water onto the loader. From the menu bar select **Cytometer > Daily Clean.**

Create an Experiment

Before running samples create your experiment in the Experiment workspace.

☑ To open the Experiment workspace, click Experiments on the Navigation bar.

☑ Use the Manage Experiment tab to create a new experiment, open an existing experiment, or to create an experiment from an existing assay.

☑ Double click a tube to select **Tube Properties**. Add and remove or modify parameters (detectors) as needed.

Important: Always remain 3 fluorescent parameters in total for the compensation matrix.

☑ Install a sample tube onto the cytometer. **Click Preview** to acquire data.

☑ Adjust the PMT voltages as needed to put all populations on scale. To adjust PMT voltages click the PMTV button in the power-left corner of the plot to enable the data sliders.

☑ *Note: A blue star in the tube properties panel indicate that settings have been modified. As PMTVs are adjusted, BD FACS Suite software automatically adjusts spillover values.*

☑ Create plots, gates for your scatter and fluorescence parameters in the worksheet as needed.

Dot plot   Histogram plot Contour plot   Density plot

☑ Double click a tube to select **Tube Properties**. In the Tube properties set

✓ **Acquisition Criteria** including time, stopping rules and gate criteria as needed.

Important: Add an additional stopping time to make sure you do not run out of sample. This is an important step, as insufficient stopping criteria will introduce air bubbles into the system.

✓ **Threshold** as needed.

☑ Use the Acquisition Status panel to select

✓ **Flow rate**. High (120 µl/min), Medium (60 µl/min), and Low (12 µl/min).

✓ **Events to display.** Select maximum number of events to display in the plot

✓ **SIT Flush.** Increase the number of flushes when extra cleaning is required to reduce sample carryover.

☑ Include a statistics and a hierarchy as needed.

☑ Create and format an experiment **Analysis Report** if needed.

Create an Experiment Analysis Report

Reports are similar to worksheets in functionality. However, only reports can be exported as PDFs after acquisition. Reports are associated and saved with the current experiment.

☑ In an experiment, click the Add Report tool on the Worksheet toolbar.
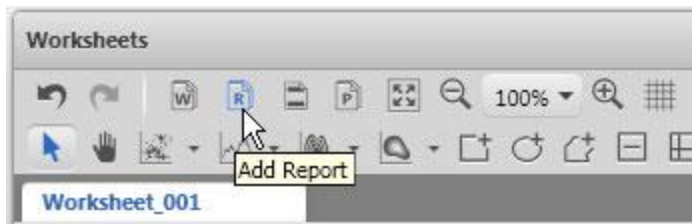
☑ Create plots, statistics views, and other analysis elements or copy/paste the elements from your worksheet. The report is automatically saved with the experiment.

Create a User-Defined Assay

Save your experiment as a user-defined assay to acquire your experiment on the loader.

☑ Create or open an experiment in the Experiment workspace.

☑ If any adjustments were made to PMTV (indicated by a blue star in the tube properties), create user-defined **Tube Settings** before creating your Assay. To create new tube settings:

✓ Prepare a tube of FACSuite CS&T beads and place it on the manual tube port.

✓ Right-click the tube that was previewed and select **> Create Tube settings**.

Note: Select Preview to view your sample while modifying the settings. Be sure not to acquire data in the tube before creating your tube settings. Tube settings cannot be created from a tube that contains saved data.

☑ From the menu bar, select **File > Create Assay**.

☑ **Click OK**. The user-defined assay is added to the library.

Experiment Acquisition in the Worklist

Using the worklist, you can acquire Assays on the BD FACS Universal Loader and export data automatically based on your preferences.

☑ Use the Worklist Symbol   in the navigation bar.

☑ On the menu bar, got to **Tools > Preferences >Worklist**. Specify General export, and print preferences for the worklist workspace.

☑ Use the Manage tab to create a new worklist: **File > New worklist** or open existing worklist.

☑ In the **> Loading options** panel, select a carrier type.

☑ Use the **> Task** panel to create worklist entries by selecting User-defined assays to add to the worklist. In the number field, enter the number of task you want to add and click Add.
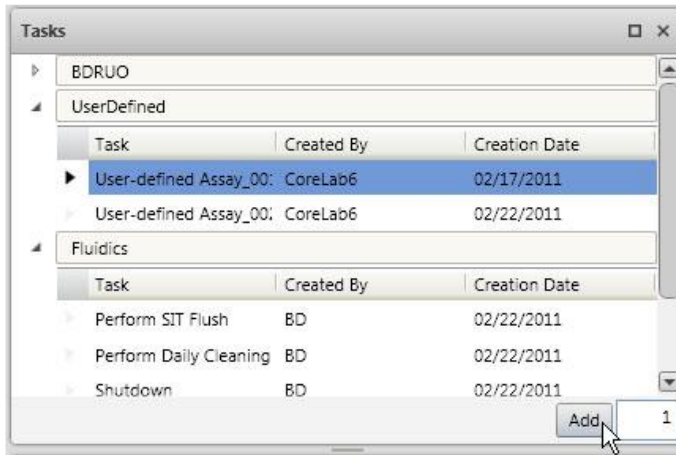
☑ In the layout view, right-click and select **> Display Properties**.

☑ Specify layout options on the General tab. Choose the location of the notch placement.

☑ Specify mixing options on the Mixing tab.
Note: Select Apply to all to apply layout and mixing options to multiple carriers.

☑ Start acquisition, click **Run all.**

☑ *Optiona*l. Click **Stop Timer** to manually stop the acquisition if you need to adjust PMT voltages, thresholds or modify gates. During preview mode, you can adjust threshold and PMT voltage and the system will automatically adjust spillover values for the current worklist only. You can also add or remove parameters before you run a worklist.

If you make changes, the Preview dialog opens. Select the third option if you want to apply these changes to all tubes, and then click OK.
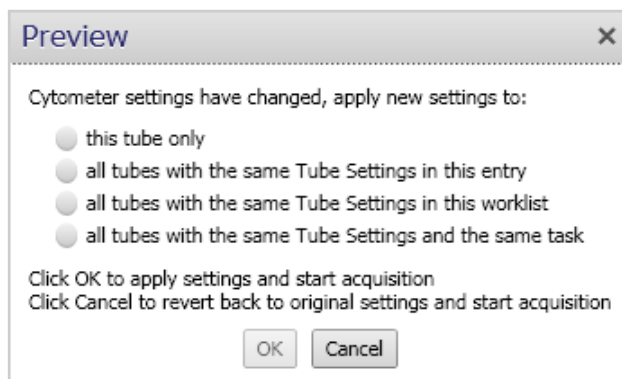
Shutting Down the System

☑ Prepare a tube with 2 ml of FACS Clean and a tube containing 3 ml of DI water.

☑ Place the tubes in the 40-tube rack (plates cannot be used).

☑ From the menu bar, select **Cytometer > Daily Clean.**

☑ Turn off FACSLyric™ power using **Cytometer > Shutdown.** Click **Ok.**

☑ Leave a tube containing 2 mL of DI water on the manual tube port.

Note: A tube containing 2 mL of DI water should be loaded on the manual tube port whenever the system is not in use.

☑ Close all open experiments, log out of the FACSSuite software **File > Exit.**

☑ Clean external surfaces and dispose used cleaning materials in biohazard.

☑ Provide information about your session in the Logbook.