

LSRII Start-Up

The complete LSRII instrument manual is located next to the LSRII computer in a blue folder. Don Foster is available in TAC S631 or at 785-7958 for help. You may also call BD at 1-877-232-8995 for assistance.

1. Turn-on the LSRII and the workstation at least 30 minutes prior to using if they are not already on. The lasers require 30 minutes to warm-up.
2. Please check the metal sheath tank beneath the LSRII and make sure that the sheath tank is filled to the bend in the tank at the top. Do not fill the tank to the very top it will not pressurize correctly.
3. Make sure the waste container is empty except for 300-500 ml of Clorox.
4. Login to the workstation using the password you received during training.
5. Open the FACS Diva software icon located on the desktop. When prompted select your user ID from the drop down list and type in your password.
6. Create a new experiment by selecting the experiment icon out of the browser toolbar
7. In the instrument panel select the Parameters that you will be using in the experiment. You can add or delete parameters using the Add and Delete buttons at the bottom of the panel.
8. Once you have all the parameters listed you may select the Instrument Menu key ->Instrument Setup ->Create Compensation Tubes.
9. In the browser you will notice a new Compensation Specimen has been created in you folder. It includes compensation tubes for all of the fluorochromes you will be using in the experiment. Select all of the fluorescence histograms while holding the shift key (this will put handlebars around all of the fluorescence histograms. Next enter the Inspector dialogue box (lower center of workspace). Select the show grid box and change the histograms background color to a color other than black. Now place a tube of unstained cells on the sample port and select the Acquire radio button from the Acquisition Controls.
10. Adjust the FSC and SSC voltages to place your desired population on screen. Adjust the fluorochrome voltages to place the unstained peaks in the first decade (the negative decade). Press the Record button in the Acquisition Control bar. Once the tube has been recorded press the Next button.
11. Unload your unstained tube and place a tube of single stained cell on the port. Load the cells and press acquire. Make sure that the P1 region is around your desired population. Place another gate (P2) around the positive population on your histogram. Adjust the stopping gate in your Acquisition control box to P2 and the events to record to 10,000. By recording 10,000 positive events you will get a more accurate compensation matrix. Repeat step 17 for however many fluorochromes you are using.
12. Once you have collect samples of all of your single color controls and placed a gate around the positive population, from the Instrument Menu at the top of the workspace select -> Instrument Setup -> Calculate Compensation. You will then have to give the compensation for this experiment a name (always use your name and then the date) and then click OK.
13. Now open a worksheet and create the plots needed to analyze your experiment. Load your tube and then press Record once you begin to see events in your plots or histograms.

LSRII Troubleshooting

| <u>Observation</u> | <u>Possible Causes</u> | <u>Recommended Solutions</u> |
|---|---|--|
| Sample tube not fitting On Sip | Sample Tube other than BD Falcon tubes used | Use BD Falcon 12X75-mm sample Tubes |
| | Worn Bal Seal | Replace Bal Seal |
| Rapid sample aspiration | Support Arm to the side | Place support arm under the Sample tube. |
| No events in acquisition Display and green RUN Button | Threshold not set to correct parameter (usually FSC) | Set the threshold to the correct parameter for your application |
| | Threshold level too high | Lower the threshold |
| | PMT voltages for threshold Parameter set too low | Set the PMT voltage higher for the threshold parameter. |
| | Air in the sheath filter | Purge sheath filter |
| | No sample in tube | Add sample to tube or install a new sample tube |
| | Sample not mixed properly | Mix sample to suspend cells |
| | Waste tank full | Empty waste tank |
| | PMT voltages set too low or too high for display parameter | Reset PMT voltages |
| | Too few events to displayed | Increase the number of events to display |
| Sample injection tube Clogged | Remove the sample tube to allow backflushing; then run a tube of warm DI water for 20 minutes | |

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| | Laser warming up | Wait the recommended amount of time for the laser to warm-up 30 min for the 488 nm (blue) 30 min for the 355 nm (UV) 15 min for the 405 nm (violet) 20 min for the 633 nm (red) |
| No events in acquisition Display and orange RUN button | RUN not activated | Press the RUN button |
| | Sample tube not installed or not properly seated | Install the sample tube correctly on the SIP |
| | Sample tube cracked | Replace the sample tube |
| | Sheath container not pressurized | Ensure that the sheath container lid and all connectors are securely seated Inspect the o-ring and replace if necessary |
| | Air leak at sheath container | Ensure that the sheath container lid and all connectors are securely seated |
| | Air in sheath filter | Purge the sheath filter |
| No fluorescent signal | Incorrect fluorochrome assignment | Make sure the Instrument Configuration in the software matches the optical filters in the instruments |
| | Wrong filters installed | Make sure the appropriate filter is installed for each fluorochrome |
| | Laser not functioning | Verify laser is malfunctioning by changing the threshold to an alterate laser while running appropriate QC beads |
| High event rate | Air bubble in sheath filter | Remove air bubbles |

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| | or flow cell | |
| | Threshold level too low PMT voltage for threshold parameter set too high | Increase the threshold level Set PMT Voltage lower for the threshold parameter |
| | Sample too concentrated | Dilute the sample |
| | Sample rate set on HI | Set the sample flow rate to MED or LO |
| Low event rate | Threshold level too high | Lower the threshold level |
| | PMT voltages for the threshold parameter set too low | Set the PMT voltage higher for the threshold parameter |
| | Sample not adequately Mixed | Mix the sample to suspend cells |
| | Sample too dilute | Concentrate the sample or increase the flow rate |
| | Sample injection tube clogged | Remove the sample tube to allow backflushing; then run a tube of DI for 20 minutes |
| Erratic event rate | Sample tube cracked | Replace the sample tube |
| | Bal seal worn | Replace Bal seal |
| | Sample injection tube clogged | Remove the sample tube to allow backflushing; then run a tube of warm DI water for 20 minutes |