

Basic Analyzer Troubleshooting

Always tell us if there are ever any problems with the analyzers, even if these steps resolve it.

The RUN button stays yellow/software says it is NOT READY

1. The FACSCaliburs need 10' & the LSR II needs 30' to warm up - have you waited that long?
2. Is the sheath tank pressurized? On the FACSCalibur, check the pressure switch.
3. Is the sheath tank able to pressurize?
 - a. On the FACSCalibur, use the switch to depressurize the sheath tank, reseal the sheath tank connector and metal cover, and then reseal everything.
 - b. On the LSR II, depressurize the sheath tank, remove the lid, and reseal the lid.
4. Are the tanks properly put in? If the sheath tank is not placed in correctly, it will not pressurize. Depressurize the sheath tank, reseal the sheath tank connector and metal cover, and repressurize the sheath tank.
5. Is the sheath tank empty/waste tank full? Fill the sheath and empty the waste.
6. Is your tube cracked? Put on a new tube of water and check again.

I don't see any events!

If this is your first sample:

1. Open a CellQuest template - any will do. Set the cytometer to RUN and press Acquire in the software. Take the tube off the sample probe, and hit PRIME. You should see lots of events. If you see no events, the computer is not connected. Restart the computer.
2. Did you check your settings? Make sure you:
 - a. Are using "Acquisition" dot plots, not "Analysis" dot plots (FACSCalibur ONLY)
 - b. Are looking at one dot plot showing all (ungated) events
 - c. Have your Threshold set on FSC at no more than 100 (FACSCalibur) or 12000 (LSR II)
3. Turn both the computer and cytometer off. Then turn on the cytometer, wait a minute, and turn on the computer. Try again from step 1.

If this is not your first sample, you may have clogged the machine.

1. Did you filter your sample? *Always filter your sample with a minimum 70um mesh!*
2. Run a tube you previously saw events in.
 - a. If you see events, then something is likely wrong with the latest sample. Run water through the machine, filter your sample, and try running it again.
 - b. If you don't - you have probably clogged the FACSCalibur and must unclog it.

If the FACSCalibur is clogged:

1. Remove any tubes from the sample probe.
2. Press PRIME. Wait for STANDBY to turn orange.
3. Press PRIME again (and wait for STANDBY to turn orange again).
4. Try rerunning a (freshly filtered) sample. If that still does not work:
5. Pour a NEW tube of 10% bleach (**Red bottle**) and RUN it on HIGH for 5'. Mark the tube and watch the volume - it should decrease.
6. If the volume drops, pour a NEW tube of Water (**Blue bottle**) and RUN it on HIGH for 5'.
7. Then try your (freshly filtered) sample again.
8. If that still does not work, ask for help! If we are not available, email us IMMEDIATELY at crm.flow.cytometry@gmail.com and leave a note on the computer for the next user.

If you have any other problems, please ask for help before trying anything else!

Remember - if you can see *anything* in the tube, it can clog the analyzers.

Always filter samples just prior to running!

Questions or problems? Just ask!

E-mail crm.flow.cytometry@gmail.com

or visit us in room 4310, Monday-Thursday 9AM-10PM, Friday 9AM-5PM.