

Current versions of the manual and the FC software are available at:

<https://www.dropbox.com/sh/zr0c17fvp50mceo/AADpL5OZxseNdaTwbM39yeXPa?dl=0>

For TRIAX SOFTWARE v.1.28 and above

THE BASIC RUN

You will need to read the PGF/Station manual to fully understand its functions. Below is an abridged list of steps that are detailed in the manual. (pg. 18) refers to the page number in the PGF manual.

10.2.1 Preparing for a run:

- 1) Turn the Gilson collector on and make sure the waste tubing is connected to a large vessel.
- 2) Turn the Frac ON and press SCAN. Wait until the Frac display reads

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- 3) Turn the PC on and boot up the Triax software by clicking the Flow Cell icon on the desktop. Work through the USER, CALIBRATE, LED SETUP and SCAN SETUP to the GRAPH page with the OD scrolling across the screen in Sample mode.
- 4) Fill the 60 cc reservoir on the right side with filtered dH₂O and prime the rinse pump with the toggle switch down (pg. 28). Flip the Rinse toggle switch up and press the RINSE key on the keyboard to rinse the system clean.
- 5) **Open the brass needle valve controlling the air flow**, press the AIR key for 10-15 sec to dry the tubing, then **close the needle valve** to prevent siphoning of air into the sample line during a run (pg. 42).
- 6) If you plan to visualize the bands using the light scattering system, fill the tube holder with water to the appropriate level using the water adjust cannula in the kit (pg. 28).
- 7) If 20 min or more has elapsed since you ZEROED the flow cell, and you are on the GRAPH page, press the ZERO (F2) button on the screen and introduce pure distilled water into the flow cell by a) applying the Rinse Adaptor (RA) to the piston and forcing water into the flow cell with the syringe, or b) exchanging the reservoir for a 20 or 30 cc syringe filled with this water and forcing it through the rinse system or c) fractionating a tube filled with this water. When the OD on the graph is stable and shows no reaction to changes in the flow velocity, complete the zero process on the screen.

Note: using the Rinse pump to force reservoir water through the flow cell leads to OD artifacts unless the rinse water has recently been boiled or degassed under vacuum. Water forced through the system with a syringe or by fractionation will give the best zero value. Noise should be in the .000x decimal

- 8) If you have just zeroed with water, open the AIR valve and dry the system with a 10 sec of high pressure air. Close the Air valve. **This step is critical for a complete run since any water reaching the flow cell ahead of the gradient will trigger the start of the run prematurely.**
- 9) Apply the **dry** tube holder cap to the top of the centrifuge tube and lock it into the holder (pg. 33). If you can see your bands with scattered light and want a permanent record of their positions and relative intensities, place a piece of tape down the right side of the holder's front window and **Mark the meniscus** and bands with the cursor in the kit (pg. 24).
- 10) Screw the clean, **dry** piston tip on the end of the piston. **Do not over tighten.**
- 11) You are ready to start. Press the START SCAN (F1) key on the PC Graph page. Do the 10 second drying if needed, then press OK. This will move the collector to the START position. The piston will then descend rapidly until it reaches the tube, slow down to the speed you designated and begin fractionation. The GRAPH will change to mm along the X-axis. As soon as the meniscus reaches the flow cell, data acquisition will commence. When this happens, you will notice the cursor shift back to 0.00 mm.
- When to abort a run:** When you press the START SCAN button, it changes to a STOP SCAN button, ready to abort if there is a software failure.
1. If the collector fails to advance when you press the OK key on the 10 s Air window
 2. If the piston does not change speed at the top of the centrifuge tube.
- 12) Each fraction advance pulse sent from the PC to the Gilson collector is marked on the graph if you have the Show Event Marks button selected.
- 13) When the tip reaches the bottom limit switch, the piston will withdraw to the upper limit switch. When the piston reaches the upper limit switch, a final pulse is sent to the collector to prepare for recovering the last part of the gradient still in the sample tubing and flow cell. It will ask you to open the Air needle valve and press the AIR button on the Fractionator. So, your number of fractions will be one more than you entered on the scan setup page.
- 14) The GP software will ask whether you want to SAVE the run and you can save it to a folder as a .csv file, which Excel and most spreadsheet programs can open directly.
- 15) If you want to do another run, Press the **NEW RUN?** button on the Graph page and confirm whether you want to do another identical run or not. If **YES**, then the Graph page reappears waiting for the START SCAN button to start.. Repeat the setup starting on step 2) above. If you want to change any of the run parameters, Click **NO** and go to the SCAN SETUP page or the USER page.