

BioComp Gradient Station Fractionator and Collector

Model 153

Step-by-Step Instructional Manual for Certified Instrument Users



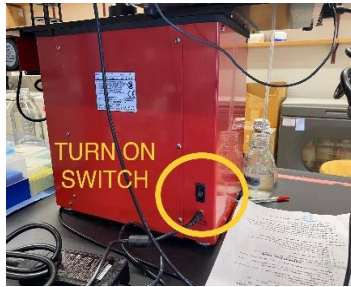
Columbia University

Precision Biomolecular Characterization Core Facility (PBCF)



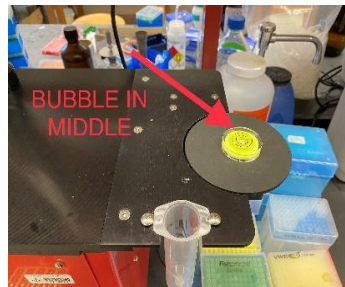
Martin Acosta (ma4160) or Jerry Chang (cc5007) for questions, concerns, emergencies.

Step 1: Gradient Maker Settings and Making Gradient Solution



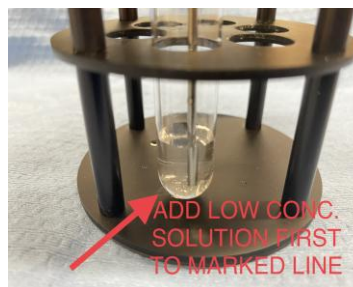
- Turn on gradient station using the switch on the back.

- On the Gradient Station panel, click on **GMST** (gradient maker station).



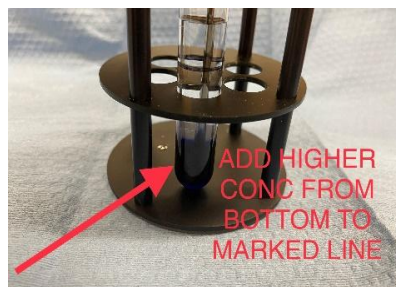
- Use the panel buttons to tilt the gradient surface **UP** or **DOWN**. Once finished, press **DONE**.

- Use a level to place the bubble in the middle, this indicates the surface is flat.



- Using an SW41 rotor, insert a tube (Seton 14x89 mm) and use the upper ridge (use short cap on tube) to mark a line around the tube. It helps to turn the tube while keeping block stationary. Place tube in tube holder. The lower ridge is meant for the longer cap.
- Using a syringe attached to a metallic long tip, place the tip at the bottom of tube and slowly inject the lower concentration up to the marked line. Be sure to remove any air inside metallic tip before injecting.
Fill 1 cm above the marked line.

Step 1: Gradient Maker Settings and Making Gradient Solution

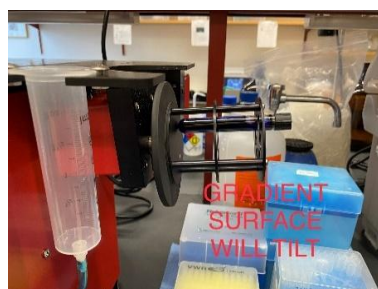


- Insert and inject the higher concentration solution at the bottom of the tube, being careful to not form bubbles or disperse drops in the lower concentration. Wipe down metallic tip to prevent addition into lower concentration solution.
- While injecting, slowly raise the tip to inject 1 cm below the separation line (dyed solution helps). Once finished, place needle tip against tube wall, and slowly raise until out of solution. It helps to pull the syringe plunger slightly to prevent drops from escaping.



- Cap tube: pivot cap into tube so the side with the hole is last to seal. Gently remove traces of liquid above cap. No air should be present inside tube.

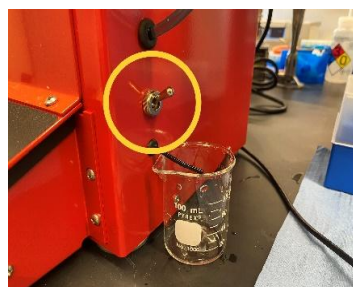
- Carefully place tubes on tube holder without tilting to sides, place tube holder on gradient surface to magnetize and secure in position. Counterbalance tube holder if using multiple tubes.



- On the gradient station panel, click on **GRAD**, then **LIST**, select **SW41**. Then select the range of concentration *such as* **SUCR 10-50%**, then click **USE**. Finally click **RUN**.

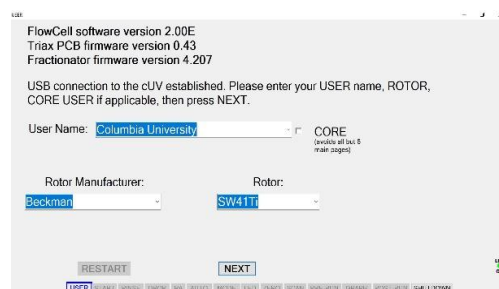
- The gradient surface will tilt at an angle, and rotate for some minutes to create a uniform gradient.

Step 1: Gradient Maker Settings and Making Gradient Solution



- Once the uniform gradient is formed, turn off the BioComp Gradient Station using the switch in the back. Then turn on again. Turn on computer with USB to BioComp connected, then press **SCAN** on the gradient station panel. Start **TRIAX** software. Wait 5 seconds.
 - Turn on **Gilson collector** using switch on back.
 - Set code on collector: 19 yields 20 samples, 30 yields 60 samples. Press **START** button to see collector path to assure this aligns with placement of sample tubes otherwise you will lose your samples!
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- Fill reservoir with DI or Milli-Q water.
 - Toggle rinse switch *up* to rinse within gradient station.
 - Toggle switch *down* to purge into glass beaker.
 - Use panel button **RINSE** to push water and **AIR** to dry system lines.
 - Perform a 3 cycle 7 second **RINSE/AIR** purge to remove anything within lines.

Step 2: TRIAX Software and Starting Run

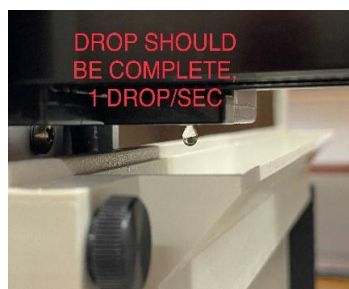


USER TAB:

- Username: **COLUMBIA UNIVERSITY**
- Rotor manufacturer: **Beckman**
- Rotor: **SW41Ti**
- Core: skips non-essential steps

START TAB: SKIP.

RINSE TAB: SKIP.



DROP TAB:

- Use the **RINSE** button to fill the lines with water, and press **AIR** to create droplets at the end of the tubing.
- To achieve a drop rate of 1 drop/sec, adjust brass air pressure knob by turning it to the right to close it all the way. Then, slowly open the knob to get 1 drop/sec, use **AIR** at end to remove all water.

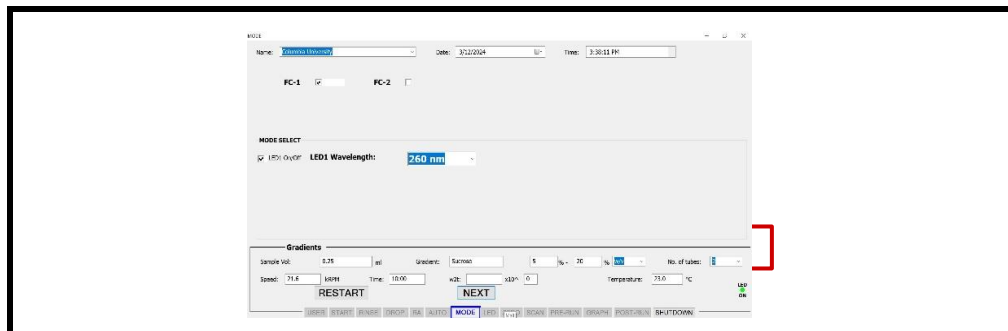


RINSE ADAPTOR (RA) TAB:

- Attach rinse adaptor to 10-20 mL syringe filled completely with DI water (no air/bubbles). Hook RA to piston and forcefully inject 4-5 mL. Leave piston attached and resting on surface.

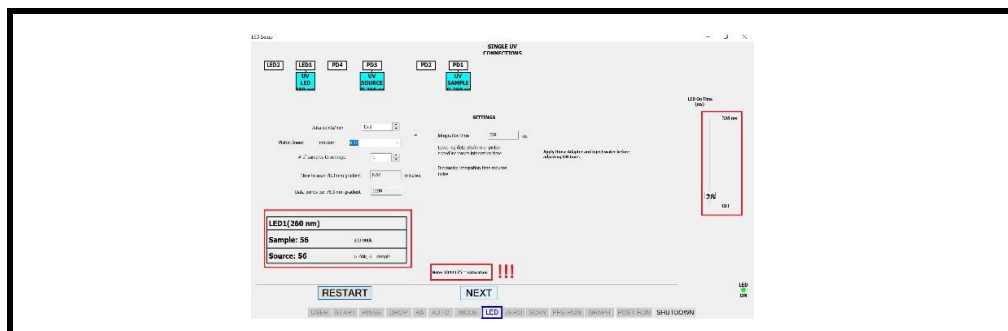
AUTO TAB: SKIP. Preset RINSE/AIR cycles to clear lines from previous runs.

Step 2: TRIAX Software and Starting Run



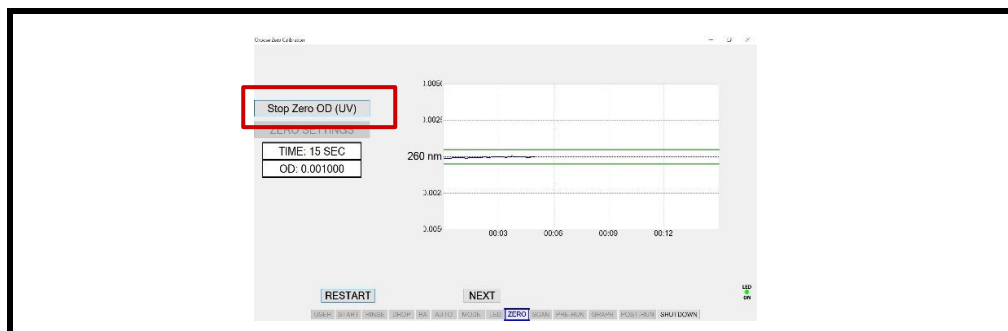
MODE TAB:

- Control detector LED set at wavelength 260 nm.
- Change percentages and indicate number of tubes as necessary.



LED:

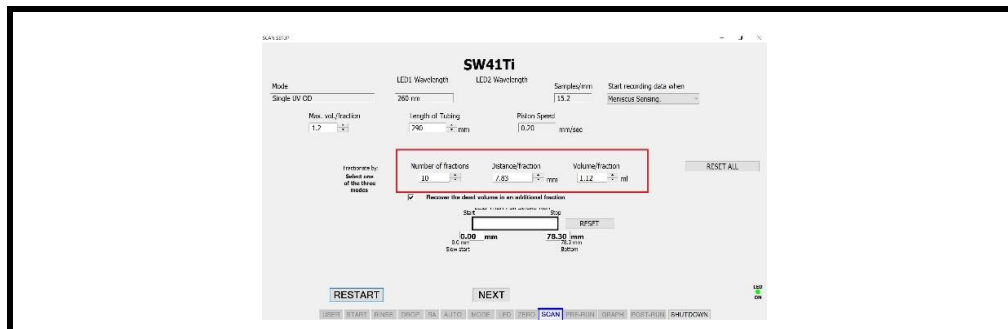
- Achieve sample (800K-900K) and source (>100K) signal by adjusting **LED ON TIME (ms)**, increasing will increase signal.
- !!! Do not surpass the saturation limit as this could damage the detector(s).



ZERO:

- Use the rinse adaptor to inject water without injecting air, assure the syringe is motionless during injection while recording zero. Signal should be within tolerance limit for ~15 seconds. Press **STOP ZERO OD (UV)** to begin.

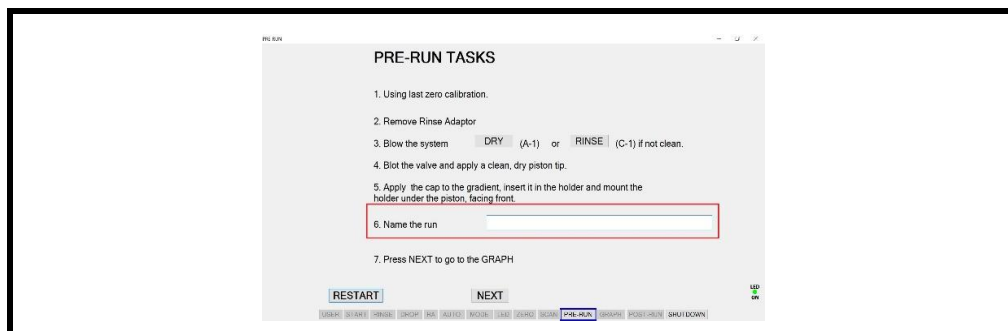
Step 2: TRIAX Software and Starting Run



SCAN:

• Settings to change number of fractionations. Distance per fraction and volume per fraction will adjust based number of fractions.

• DO NOT change any settings outside the red box.



PRE-RUN:

• Remove RA.

• Blow the system using DRY button on gradient panel.

• Blot valve where RA was attached and careful apply a clean piston tip.

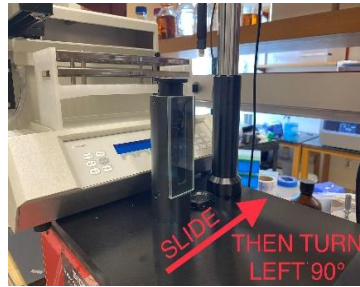


• Carefully remove cap from gradient tube.

• Carefully apply piston cap to gradient tube without tilting tube.

• Place gradient tube inside piston holder and install it under the piston by sliding it through the slit and rotating the transparent surface towards the user.

Step 2: TRIAX Software and Starting Run



- Place gradient tube inside piston holder and install it under the piston by sliding it through the slit and rotating the transparent surface towards the user.



GRAPH:

- Press **START SCAN** to begin analysis.

- Optional: press **REFRESH** to clear the graph, and **PAUSE** to temporarily pause the detector.

- Shutdown Instructions
1. Remove the piston tip, rinse it with dH2O and vigorously shake it dry.
 2. Blot the bottom of the valve to remove water under the ball valve in the cap.
 3. Press the RINSE key (2) for 5 sec.
 4. Dismount the Reservoir, pour out the water and unscrew it from its lure fitting.
 5. Locate the AIR 60 cc syringe and pull its plunger back but not out.
 6. Attach the AIR syringe to the rinse tubing lure fitting.
 7. With the Toggle switch DOWN, forcefully push the AIR plunger and blow the tubing and pump dry.
 8. Slowly pull the plunger back and forcefully push it in. Repeat up to 3 x.
 9. Pull the plunger back, Toggle switch UP and forcefully push it in to force air through the rinse system.
 10. Toggle DOWN, pull plunger up to refill with air, Toggle UP, push forcefully. Repeat 3 x.
 11. Replace the Reservoir syringe on its luer fitting and mount it on the right wing.
 12. Blot the bottom of the piston to remove any water left in the valve cap.
- STERILIZE DONE
- LED ON

SHUTDOWN:

- Follow the instructions on the SHUTDOWN menu.

- STERILIZE after 3 runs, or as necessary, execute sterilization to remove residual sucrose using ethanol.