BioComp Gradient Equipment Instruction

Rotor SW41, Centrifuge Tube: SECTON 7030

Laying gradients

1) Mark the halfway point of the tube using the marker block with the upper edge (short caps)
2) Light solution
    a. Suck the light solution into a syringe (> 10 mL) with the provided cannula
    b. Lay the light solution in the tube slightly above the line
3) Heavy solution
    a. Suck the heavy solution into a syringe
    b. Clean the outside of the cannula with paper towel
    c. Push a drop of liquid out of the syringe and touch the cannula to paper tower to remove the excess solution
    d. Push the cannula down the bottom of the tube along the wall
    e. Laying the heavy solution and move the end of cannula upward to keep it 1 cm below the interface
    f. When the interface reaches the line, move the cannula to the wall and pull the cannula out.
4) Cap the tube
    a. Pivoting the cap into the tube so the side with the hole is the last to seal
    b. Liquid should be visible inside the cap
5) Form the gradient
    a. Turn on the system from the back
    b. Select GMST
    c. Use the UP and Down buttons to level the plate
    d. Put tubes in the tube holder on the Gradient Master
    e. Choose your gradient by selecting SW41 rotor; finding the gradient in the list; and hitting Run. Making a 10-50% gradient takes around 3 min
    f. No bubbles in the gradient!!! If there are bubbles, remake the gradient.

Users need to clean cannulas right after making gradients.

Loading samples

1) The gradient should be kept at 4 ^{o}C for at least 45 min before laying samples
2) Take the gradient cap off the centrifuge tube from the hole side first
3) After taking the cap off, the meniscus is about 3 mm below the opening. You should leave the **2 mm empty space after loading**. Therefore, you only can add 1 mm liquid, around 150 µL. To add more sample, you need to pipette out liquid from the top layer. For example, you want to **load the max amount 500 µL**, you **need to firstly pipette out 500-150=350 µL**.
4) Balance two tubes after pipetting out liquid.
5) After loading samples, confirm the weight difference, which should be less than 0.1 g.
6) Clean tubes outside before loading to a bucket

Users need to clean gradient caps right after starting centrifugation.

Centrifugation

Follow your procedure to centrifuge samples

1) The balanced two tubes should be loaded opposite to each other on the rotor
2) All six buckets should be loaded to the rotor

Fractionation

1) Fill up the rinse reservoir with MillQ water
2) Prime the pump
    a. Push the toggle down and wait for water coming out of the waste tubing
    b. Flip the toggle up
3) Turn the machine off and turn it back on
    a. Hit the scan mode
4) Launch the software
    a. Check the Core box
5) Turn on the fractionator
6) Screw the rinse adaptor to the piston
    a. Push 5 ml water into system
7) Input Gradient information
8) Adjust the absorbance signal
    a. Add water to the flow cell (Already done)
    b. Adjust the On time to make the sample value between 800 K and 900 K
    c. The On time should be change between run to run. If values change, the cell might not be cleaned well.
9) Zero OD (Only need Zero once a day)
    a. Click the Start Zero OD button
10) Define the number of fractionating tubes
    a. Select Meniscus Sensing
    b. Change the collecting volume of each tube
11) Pre-Scan
    a. Remove the rinse adaptor
    b. Install the **piston tips (need to be cleaned and dried between runs)**
12) Mount the tube in the holder
    a. Push the mount cap on the tube and give a twist. The tube should be securely attached to the cap
    b. Load the cap and the tube in to the mount.
    c. Align the line on the cap with the screws on the mount and twist the cap clockwise to lock it
    d. Slide the mount on the top of the light and twist clockwise
13) Name the run
14) Set up the fractionator
    a. Load empty tubes on the tube rack
    b. <30 tubes use the code 19 and load empty tubes to the middle two lanes,

    c. >30 tubes use the code 29 and load empty tubes to four lanes
15) Start scan from the software
16) Piston goes into the tube and run starts
17) Save Run

Cleaning

1) Click the sterilization button
2) Rinse the piston
    a. Take out the piston tip and connect the rinse adaptor
    b. Wash the piston with 5-10 mL water by pushing the water through the adaptor
3) Rinse the system
    a. Fill the rinse reservoir with MillQ water
    b. Clean the system by holding Rinse and Air each 5 sec for 3 times
        i. (Rinse 5 sec + Air 5 sec) X 3
4) Sterilize the system
    a. Empty water inside the rinse reservoir and fill it up with 15 mL 70% Ethanol
    b. Rinse the system with 70% Ethanol
    c. Hold air button for 5 sec
5) Air the system
    a. Disconnect the rinse reservoir and connect an empty 60 mL syringe with the plunger pulling to the end
    b. Air the pump
        i. Push the toggle switch down
        ii. Push air into the pump
        iii. Retract the plunger and push air several times
    c. Air the Triax
        i. Push the toggle switch down + Retract the plunger
        ii. Push the toggle switch up+ Push air
        iii. Repeat step i and step ii three times