Preparation for gradient separation with computer controlled pumps

This instruction is replacing AL5178 ”Förberedelse för gradientseparering med datorstyrda pumpar. Preparation for gradient separation with computer controlled pumps 710964” because that instruction broke and can not be updated anymore.

Safety routines

All work with human material carries the risk of transmitting infectious diseases. See Skyddsföreskrift laboratoriearbete KITM AL4731.

Procedure

Preparation

Solutions

- Mark 15 black capped T-25 flasks from 1-15 per planned purification. Fill flask 4-14 with 30 ml wash solution. Keep the flasks cold (refrigerator) until gradient purification collection.

COBE

- Make sure that the COBE cooling system is connected to the COBE and start the cooling system. Start the COBE cooling system with the POWER button.

Procedure

1. Open the the right (höger) and left (vänster) COBE bag set, or the one that is needed. Clamp all the colored tubes close to tubing manifold and ensure that the clamps are securely attached.
2. Place the COBE bag(s) into the COBE machine.
3. For the COBE on the left the yellow tubing should be drawn into the hood via the holes on the side of the biological safety hood. For the COBE to the right it is necessary to wait until the density gradient tubing has been mounted before the blue tubing can be placed in the hood.
4. Turn on the power strip for the pumps. The speed should be set between 100-300. If it is not set, set it to between 100-300 by using the arrows below the LED numbers.
5. Twist off the plastic lid for Pump 2 or 4, light density gradient pumps.
6. Mount the pump tubing attached to the pink tubing into the notch closest to the LED display. Make sure the arrow pointing to the right is chosen.
7. CAUTION! Mind your fingers in this step. Press the ON/OFF button to start the pump. Set in the tubing carefully in between the pump rim and the pump heads at the same time pressing down on the tubing and the pump heads. If they are not set properly, the plastic pump lid will not go back into position. Make sure the metal part of the rollers lay flat against the bottom of the pump head plate.

8. Press ON/OFF to stop the pump and mount the pump tubing in the notch furthest from LED display.
9. Place the plastic lid back onto the pump 2 or 4. The notch in the lid aligns with the small metal bead in the pump itself.
10. Repeat steps 4-9 with the pump tubing connected to green COBE tubing in pumps 1 or 3, heavy density gradient pumps.
11. Turn on the computer and log in.
12. Open PNET.
13. Go to “File/Open…” and open “Configuration to run COBE”. The file can be found in “Mina document/Automated Pump System/”. The pumps should read “000”.
14. Press the “Opt” button for Pump 1 or 3, press “Open” and choose ” UIC-UB Pump 1 eller 3 Heavy Ficoll 280 ml Linear” and press “Open” and then “OK”. Pump profiles are found in the same folder as in step 13.
15. Press the “Opt” button for Pump 2 or 4, press “Open” and choose ” UIC-UB Pump 2 eller 4 Light UW 280 ml Linear” and press “Open” and then “OK”. Pump profiles are found in the same folder as in step 13.

Continue according to Gradientseparering med datorstyrda pumpar. Gradient separation with computer controlled pumps AL5477

Protocol and Archiving

This protocol requires the protocol Lösningsprotokoll och odlingsmedium AL6662 (Preparation of working solutions for human islet isolation) and information is archived for a minimum of 10 years.

Equipment

Apparatus

Hood	MTA 074205
Tubing pump, Watson Marlow	MTA 34505
COBE-cooling system	MTA 073079
Centrifuge COBE 2991	MTA 33876, 072198
COBE cooling system	MTA 075061, 078667
Sterile tube welder x 2	MTA 079355
Tube welding	MTA 16762
Gradientpumpar 1, 2	MTA 079354, 079353
Gradientpumpar 3, 4	MTA 078659, 078660

Material

T-flasks, Corning, black cap 25cm^2	746180
Clamp, plastic	746099
Transfer set	746098

Reagents

Biocoll 1.100 g/ml HEAVY solution	767602
UW LÄTT brukslösning	758240
Wash solution	767600
Ringer's Acetate	758238

Good to know

If the computer cannot detect the pumps, you have to assign the correct address to the pumps according to the following:

- Pull out the power supply cable from the rear of the pump.
- Press and hold the direction button (looks like this ◄I ►)while plugging the cable into the socket again.
- The front panel illuminates and the the display shows A00
- Release the direction button and select a desired address from 0 to 99.
- Press the ON/OFF button to save it to pump memory.
- The following adresses should be used:
  - Pump 1: 1
  - Pump 2: 2
  - Pump 3: 3
  - Pump 4: 4

References

Formulas

Tvättlösning (Wash solution)	767600
Biocoll 1.100 g/ml TUNG brukslösning	767602
UW LÄTT brukslösning	758240

Related documents

Skyddsföreskrift laboratoriearbete KITM AL4731

Förberedelser inför ö-isolering. Preparation before islet isolation. AL5180

Förberedelse för datorstyrd gradientseparering. Preparation for computer controlled gradient separation.

Gradientseparering med datorstyrda pumpar. Gradient separation with computer controlled pumps. AL5477

COBE 2991 Blood cell processor. AL5474

Lösningsprotkoll och odlingsmedium AL6662