Centrifugation

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I. Centrifuges in the Schepartz Lab

Each lab within the Schepartz Lab Complex has a microcentrifuge for use with 1.5 mL or 0.5 mL Eppendorf tubes. These microcentrifuges are also used for the QIAGEN Miniprep kits, etc. – anything that requires a tabletop centrifuge. Use is amazingly straightforward: place your tubes in the centrifuge in a balanced arrangement, close the top, and do one of two things: either set the timer for a long run, or press the button on the front for a moment to simply 'pulse' the tubes. For work that must be done at 4ºC, there is a microcentrifuge in the deli case in the hot room.

The deli case in the hot room and the deli case near Kamil's bench (room KCL100) also contain centrifuges appropriate for 50 mL conical tubes. Make sure your tubes are balanced, cap included, before spinning. A swinging rotor centrifuge is in Joshua's hood (K102). The rotor for this centrifuge is appropriate for 15 mL Falcon tubes.

Keep in mind that Speed-Vacs are not typical centrifuges; they are specifically for drying small samples, and procedures for speed-vacs are outlined elsewhere in this manual. For proper use of any centrifuge, keep a few simple things in mind: balance your sample tubes, clean up any messes you happen to make, and inform your TA of any problems with the centrifuges.

II. Other Centrifuges Available to the Schepartz Lab

This is a general overview of the preparative centrifuges available to our lab in the Kline Chemistry Building. Remember, none of these centrifuges belong to our group, so be conscientious when using them. Betty Freeborn in the Moore Lab is the person in charge of them. Always clean up after yourself, and log usage in the appropriate logbook. Always ask for help from someone who has used the centrifuge you need before starting for the first time.

For information on appropriate rotors and conversion information from RPG to RPM, see the Schepartz lab website directory of 'Cool Science Links' (http://www.paris.chem.yale.edu/links.html), where you will find links to Sorvall and Beckman rotor caclulators.

A. Sorvall Superspeed RC2-B

This centrifuge belongs to the Crothers group and is not an untracentrifuge. It is useful for spinning bacterial broths and working up the cells. The common rotor is the SGA rotor that accepts bottles with volumes of 250 mL each. This one is ideal if you have up to three 500 mL broths because a single spin will be sufficient to pellet the cells. There is also a GS-3 rotor that takes 6 500ml tubes. It is great for large volumes (as is the Beckman J2-21 discussed below). When using this centrifuge, remember that there is no vacuum in the chamber, so you cannot perform very high-speed spins. For the SGA rotor, and for the GS-3 rotor, you will need to get above 6000 rpm to do your work. To display the speed in "g" you can flip a switch from rpm to rcf.

Procedure:

1. Place the rotor you wish to use in the centrifuge. Set the temperature and wait for at least 30 minutes to allow the rotor to cool before you do your run.
2. Pour your sample into at least two bottles that have screw top lids. The O-ring lids work best to prevent leakage during the spin.
3. Balance the samples to within 0.1 g (including the lid).
4. Place the bottles in the cooled rotor.
5. Attach the lid with the two attachment screws in the direction shown.
6. Secure the top on the chamber.
7. Set the speed.
8. Set the time and begin your run.
9. Remove your samples when the run is complete.
10. Check carefully that the bottles have not ruptured or leaked into the rotor.
11. Remove the rotor from the centrifuge. Turn off the power when finished and leave the lid open.
12. Clean and dry the rotor thoroughly when finished. ***Do not use harsh chemicals to clean the rotors or damage will result.*** Use soap and water and a teflon brush (in the Moore's lab across the hall; ask Betty if you can't find it). Place rotor upside down on the paper towels when finished.
13. Clean all the condensed water that formed in the open centrifuge.

B. Beckman J2-21

This centrifuge belongs to the Moore Lab and is useful for the same types of runs as the Sorvall. It will accept several different rotors, including one with a maximum bottle size of 500 mL, so it is useful for large bacterial growths.

Procedure:

The procedure is the same as for the Sorvall. There is also a vacuum that must register in the green region of the gauge prior to starting your run. For the rotor that accepts the 500 ml tubes, use a special crowbar to insert a rotor into the centrifuge; the rotor is quite heavy and the inside of the centrifuge is narrow. You will find the crowbar next to the rotor (usually). The crowbar screws into the center of the rotor with the arm that has threads.

C. Beckman L-70K (and L8-70M)

This is an ultracentrifuge useful for cesium chloride gradients of plasmid preparations or ammonium sulfate fractionations in protein preparations.

Procedure:

1. Choose your rotor. **Only use rotors designed for use in this centrifuge!**
2. Cool the rotor by storing at the desired temperature for at least 1 hour.
3. Once the sample/rotor is in the centrifuge, turn on the vacuum and wait until the chamber is <100 microns. This can take awhile so you do not need to monitor it constantly.
4. Set the desired speed, time, and brake. **Do not exceed the speed ratings of the rotor!**
5. Start the run.
6. When run is complete, follow the above instructions for cleaning and storage of the rotor.
7. Turn off the instrument.