DEPARTMENT OF MOLECULAR AND CELLULAR BIOLOGY

SAFE OPERATING PROCEDURE

BECKMAN HIGH SPEED AND ULTRACENTRIFUGE

Purpose:
To provide safe operation and instructional use for Beckman High Speed and Ultra Centrifuges for research laboratory staff and students in the Department of Molecular and Cellular Biology.

Emergency Contact: Jamie Jones SCIE 4482 ext. 53816

Beckman Centrifuges for shared MCB use are located in:
SCIE 2202: Ultra L-60, L-90K, High-speed: Avanti J25 I, J2-21, J30 I
SCIE 3202B: Ultra L8-55
SCIE 4203: Ultras: L8-55M, L-100XP, Optima Max tabletop

General safety notes for High Speed and Ultra Centrifuges:

Careful centrifugation technique is essential, because forces generated in high-speed centrifugation can be enormous. For example, 10 grams at the bottom of a JA-25.50fixed-angle rotor rotating at 25,000 rpm exerts the gravitational equivalent of 0.8 ton of centrifugal mass at the bottom of the tube cavity.

- All operators must receive training prior to using the equipment. Please arrange training by contacting Jamie Jones (Department Support Technician), ext. 53816/ SCIE 4482. It remains the responsibility of the supervisor to ensure their personnel are adequately trained.
- DO NOT centrifuge flammable, radioactive or toxic materials in shared centrifuges.
- DO NOT attempt to move the centrifuge while it is in use.
- DO not operate instrument above 38°C.
- DO NOT leave the centrifuge refrigerated for prolonged periods without vacuum in the chamber. This causes condensation, which affects the performance of the vacuum pump.
- DO NOT turn off the power with vacuum still in the chamber.
- If any unusual vibrations, sounds, or odours occur, TURN THEPOWER SWITCH OFF until the cause has been determined and be sure to report the issue.
- Turn the POWER switch off when not in use to conserve energy and to avoid excess instrument wear.
- Defrost chamber and wipe up accumulated moisture; water in the chamber will prevent efficient temperature control.

Tube Inspection and Care:

- Only Beckman tubes or bottles may be used in these rotors. Tubes and bottles must be inspected before each use to make sure that they are in excellent condition. Verify that the tubes in use can withstand the g-force generated by the run conditions by cross referencing with Beckman manuals and product specifications. Small scratches in glass or polycarbonate tubes can cause failure at high g-forces, resulting in the loss of the sample, an imbalanced rotor, and potential damage to the centrifuge.
- Tubes may display crazing: small cracks that do not penetrate all the way through the wall, but if a crack approaches the outer wall of the tube, discard it. Do not use a tube that has become yellow or brittle with age. A tube may fail if it is not the correct shape, or if an incompatible solvent/tube-material combination is used (cross reference with manufacturer's instructions).
- Tubes should be 75 % filled and not overfilled. Tubes with a significant airspace may collapse during the run and become jammed in the rotor. Tubes which are half-filled or less may not be run at more than half the designated maximum rotor speed.
- Samples are always run in pairs. Tubes/bottles are weighed using a pan balance including the lids so that they are of identical weight. If running only one sample, use a second tube/bottle containing water as a balance and ensure that the weight of the two vessels is the same.

Most tubes and accessories, except those made of Ultra-Clear, polyethylene, Noryl, or cellulose propionote, can be autoclaved at 121°C for about 20 minutes. Note that autoclaving reduces the lifetime of polycarbonate tubes. Also, polyallomer tubes may be permanently deformed if they are autoclaved many times or if they are handled or compressed before they cool. Tubes should be placed open-end down or supported in a rack if autoclaved.

Do not autoclave tubes or bottles with caps on. Pressure in a sealed container can cause an explosion. Pressures within the autoclave can cause partially sealed containers to collapse when the autoclave vents.

Rotor Care:

- Choose the correct rotor specific for the centrifuge. There are different rotors that may be used in the Beckman ultracentrifuges. Fixed angle rotors are the most common; swinging bucket and vertical rotors may also be used in special applications. If unsure, reference the manufacturer's manual for information on rotor and tube selection. Check the rotor against Beckman's class rating sticker on the centrifuge.
- Always inspect and apply a thin layer of vacuum grease to the O-rings on swinging buckets, and rotor lids and check for signs of wear. **DO NOT RUN A ROTOR WITH MISSING OR CRACKED O-RINGS**.

- Check over speed disc and rotor for any signs of wear or damage. Replace if necessary and report to Jamie Jones ext. 53816, SCIE 4482.
- Lift rotors vertically to avoid bending the driveshaft.
- If you are using a rotor with six or twelve wells, you may do a run using three tubes/bottles. They must all weigh exactly the same and be in every other well. You should use a balance to weigh the tubes/bottles and be sure to include the lids when weighing them.
- Rotors are stored upside down to prevent condensation forming in the wells. Check the rotor before using it to make sure that it is clean and dry. Any liquid or debris in the wells will affect the balance of the rotor and possibly contaminate samples. Put the rotor back in the refrigerator after use. It must be stored, clean, dry and upside down.
- To clean a rotor you must wash it with Beckman Rotor Cleaning solution and water. If the rotor does not come clean with rinsing then you may need to use a rotor brush to clean it. You must use the proper rotor brush with the rubberized ends. These ends prevent the brush from scratching the rotor as this may lead to corrosion.
- Rotor care involves not only careful operating procedures but also careful attention to:
- Regular cleaning, decontamination, and/or sterilization as required,
- Frequent inspection
- Corrosion prevention
- Regular and proper lubrication.

  Do not use sharp tools on a rotor or buckets, as the anodized surfaces can get scratched. Use plastic or wooden tools to remove 0-rings or gaskets for cleaning. Corrosion begins in scratches and may open fissures in the rotor with continued use. The corrosion process accelerates with speed induced stresses. The potential for damage from corrosion is greatest in aluminum rotors and components.

Rotor types:

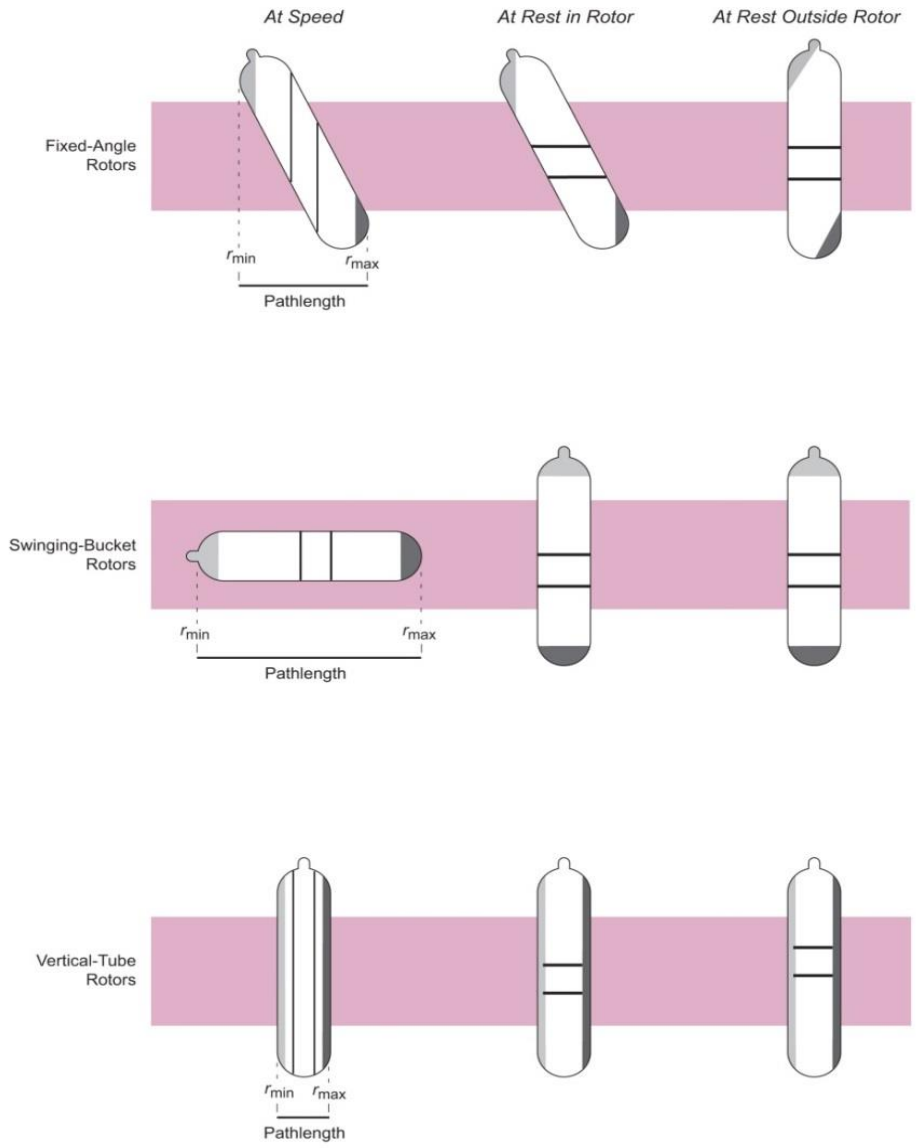
- **Fixed-angle rotors** are general-purpose rotors that are especially useful for pelleting subcellular particles and in short-column banding of viruses and subcellular organelles. Tubes are held at an angle (usually 20 to 45 degrees) to the axis of rotation. The tube angle shortens the particle path length compared to swinging-bucket rotors, resulting in reduced run times. Tubes can be placed directly in a rotor cavity if the diameters of the tube and the cavity are the same. Using adapters, more than one type and size of tube can be centrifuged together, provided that the loads are properly balanced.

- **Swinging-bucket rotors** are used for pelleting, isopycnic studies (separation as a function of density), and rate zonal studies (separation as a function of sedimentation coefficient). Large swinging-bucket rotors are used to obtain cell-free plasma or for cell packing. These rotors can be equipped with racks or microplate carriers to hold a variety of tubes, bottles, blood bags, or multiwell plates.

✓ **Vertical-tube rotors** hold tubes parallel to the axis of rotation; therefore, bands separate across the diameter of the tube rather than down the length of the tube (see Figure 1.2).'Only Quick- Seal tubes are used in vertical-tube rotors, making tube caps unnecessary.

Particle separation in fixed-angle, swinging bucket and vertical rotors:

**Dark gray represents pelleted material. Light gray is floating components, and bands are indicated by black lines.*



Reference: Beckman Coulter, Inc. PN JR-IM-10AF February 2014

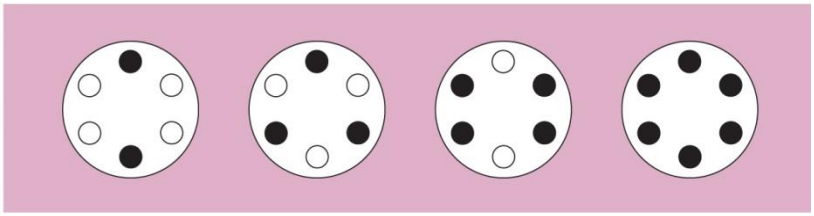
Rotor Balance:

The mass of a properly loaded rotor is evenly distributed on the centrifuge drive hub, causing the rotor to turn smoothly with the drive. An improperly loaded rotor will be unbalanced; consistent running of unbalanced rotors will reduce centrifuge drive life. To balance the rotor load, fill all opposing containers to the same level with liquid of the same density. Weight of opposing containers must be distributed equally. Place tubes in a fixed-angle, vertical- tube, orJS-24 series swinging-bucket rotor symmetrically, as illustrated.

If sample quantity is limited and the rotor is not balanced, do one of the following to balance the rotor, depending on the rotor in use:

- Load the opposite rotor cavities or buckets with tubes containing a liquid of the same density as opposing tubes.
- Layer a low-density, immiscible liquid, such as mineral oil, on top of the sample to fill opposing tubes to the same level.

Arranging tubes symmetrically in a fixed angled, vertical tube or swinging bucket rotor.



Reference: Beckman Coulter, Inc. PN JR-IM-10AF February 2014

Rotor Storage:

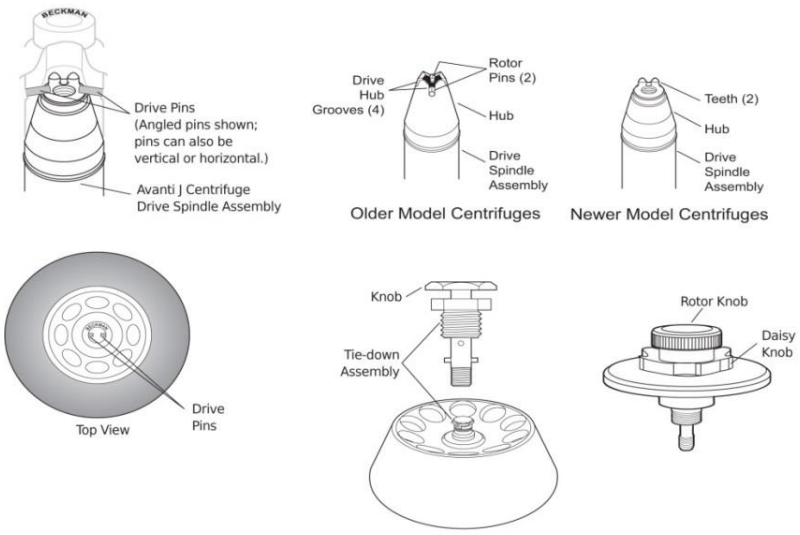
When it is not in use, store the rotor in a dry environment (not in the instrument) with the lid removed to allow air circulation so moisture will not collect in the tube cavities.

Rotor Tie-Down:

To secure the rotor to the drive spindle hub during centrifugation, J series rotors are equipped with devices that screw into the hub. If the rotor is left in the centrifuge between runs, tighten the tie-down device before each run.

- Some rotors are equipped with tie-down assemblies. These may be knobs that can be hand-tightened when the rotor is installed, and between runs if the rotor is left in the centrifuge. Other tie-down assemblies are tightened by turning the rotor lid knob.
- Some new and modified rotors have dual-locking lid mechanisms. The dual-locking lid mechanism consists of a daisy knob that secures the lid to the rotor, and a tie-down knob that attaches the rotor to the centrifuge drive hub. (Daisy refers to the knob shape. The grooves between each "petal" let your fingers grip the knob firmly and provide leverage for turning.) The daisy knob allows you to attach the lid to the rotor before placing the rotor into the centrifuge, and to remove the rotor from the centrifuge with the lid attached.

Reference: Beckman Coulter, Inc. PN JR-IM-10AF February 2014



Starting an ultracentrifuge run:

Be sure to reserve your centrifuge run in the log book and check the log for any previous issues with the centrifuge before starting. The log book must be signed when you turn on the instrument, NOT after the run has finished.

- About 10-20 minutes before the run, check that the chamber is empty. With the lid closed, turn on the vacuum and refrigeration to pre-cool the ultracentrifuge. **Never leave refrigeration on without vacuum.** Condensation will reduce the performance of the vacuum system and lead to slow pump-down times. Similarly do not leave a chilled rotor on the bench any longer than necessary to load it especially during humid weather. If you run at ambient temperature, no pre running of the vacuum is necessary.

- Set the desired run temperature, using the panel buttons.
- A sectored disk on the bottom of the rotor controls the speed of each rotor (over speed disc). Before loading up your rotor, check that the disk is in good condition and securely attached. Wipe any condensation from the rotor and tubes. Put your tubes/bottles into the rotor so that it is balanced and tubes have been pre-weighed on the pan balance. Check to ensure all O-rings are present and have a light coating of vacuum grease. If any O-rings are missing contact Jamie Jones, ext. 53816, SCIE 4482. Put the lid on the rotor and tighten the knob on the lid before installing it in the ultracentrifuge. If the rotor lid will not screw on easily, apply a thin coating of Spin Kote to the threads of the rotor lock screw. Spin Kote is only for metal components.
- When ready, release the vacuum and install the rotor by lowering it **gently** onto the drive spindle. Push down on the rotor gently to ensure that it is seated. For all except swinging bucket rotors, feel carefully under the rotor. **Gently, turn the rotor by hand to ensure that it is seated correctly.**
- Swinging bucket rotors need special attention to ensure that they are correctly mounted. **Use all six buckets for all runs even if some are empty**. Buckets and lids are numbered, and must be mounted in the correct position on the rotor. Ensure that each bucket is mounted by both hooks and is swinging freely. When mounting the rotor, be careful not to dislodge a bucket. Since you may only use visual inspection to ensure that a swinging bucket rotor is correctly mounted, it is good practice to mount the rotor without buckets before you attempt a "live" run.
- Close the chamber door and turn on the vacuum. Unless your sample is very thermally sensitive, leave the overtemp control at the default setting. If you do set below ambient temperature, do not set overtemp less than 10° above the working temperature. Select and set the run speed and time of your run.
- From the control panel, set time and speed parameters. There's an automatic start up procedure; the ultracentrifuge will only accelerate when there is sufficient vacuum and will hold at 3000 rpm until that point. However, the clock will run down while vacuum is developing, so you won't get your full run time. If you want reproducible run times, a better procedure is to wait for the vacuum to get to the first stage before trying to start (200 microns on the L-60).
- Watch your ultracentrifuge as it accelerates. Excessive vibration or noise as the speed builds up through 1500-3000 rpm may indicate a balance problem, especially with the swinging bucket rotor, and most incidents occur in this initial acceleration phase. Hit the stop button immediately at the first sign of problem. **Wait until the centrifuge has reached full speed before leaving the room.**

Stopping a Run:

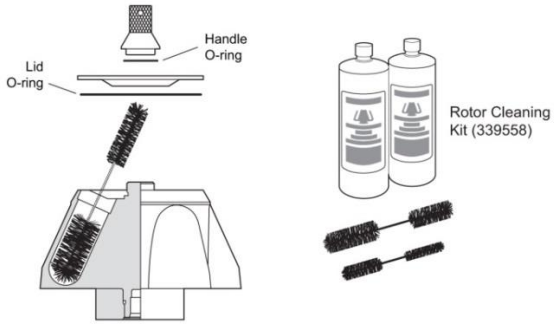
- Terminate the run either by allowing the timer to shut off automatically or by pressing the STOP button.
- Wait for the rpm indicator to reduce to zero, indicating full rotor stop, and then switch off the vacuum. Press on the ultracentrifuge door handle toward the center of the lid to slide the lid across. Lift the rotor straight up and out of the ultracentrifuge.

- Ensure that the vacuum is released by opening, then closing the chamber. Turn POWER switch OFF when ultracentrifuge is not in use.
- Check to see that the rotor is clean inside. If the rotor is not clean, wash it with the diluted Beckman Rotor Cleaner and the rotor brushes (these brushes have rubberized ends). Dry the rotor and put it back into the refrigerator upside down.
- If you have any spills in the ultracentrifuge, you must report them to your supervisor immediately. The user is responsible to clean up any spills that have occurred during the run.
- If you experience any problems with the ultracentrifuge, you must also report these to Jamie Jones, ext. 53816, SCIE 4482.
- If a tube is jammed in the rotor and does not come out easily, contact Jamie Jones for assistance. **DO NOT use any metal tools to pry the sample out of the rotor.**

Rotor Cleaning: (Beckman Coulter Guidelines)

- Wash the rotor and rotor components immediately if salts or other corrosive materials are used or if spillage has occurred. Do not allow corrosive materials to dry on the rotor.
- With normal usage, wash rotors frequently to prevent corrosion that can begin in scratches. Remove buckets from yokes before cleaning swinging-bucket rotors.
- **CAUTION**: Do not immerse or spray a swinging bucket rotor yoke (or body) with water because liquid can become trapped in the hinge pin area and lead to corrosion.

1. Remove the O-rings with a plastic tool (never metal) before washing.
2. Wash the rotor and lid in a mild detergent, such as Beckman Solution 555 (339555), that won't damage the rotor. The Rotor Cleaning Kit contains two plastic-coated brushes and two quarts of Solution 555 for use with rotors and accessories. Dilute the detergent 10 to 1 with water. **NOTE**: Do not wash rotor components in a dishwasher. Do not soak in detergent solution for long periods, such as overnight.
3. Rinse the cleaned rotor and components with distilled water.
4. Air-dry the rotor and lid upside down. Do not use acetone to dry the rotor.
5. Apply a thin, even coat of silicone vacuum grease to both lid O-rings before replacing them in the grooves in the lid.
6. Clean metal threads as necessary (at least every 6 months). (A).Use a brush and concentrated Solution 555.
   (B). Rinse and dry thoroughly, then lubricate lightly but evenly with Spinkote to coat all threads.
7. Periodically remove the O-rings and wipe clean as necessary. (A). Clean the O-ring grooves with a cotton-tipped swab. (B). Reapply a light film of silicone vacuum grease.



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Spill Clean-up:

- ✓ Spills inside the ultracentrifuge may occur from the failure of a tube, bad/ faulty O-ring seal or overfilled tube volume.
- ✓ Further operation of the ultracentrifuge is not allowed until the spill is cleaned up. Shut centrifuge off and do not open the lid for 20 minutes to allow potential aerosols to settle.
- ✓ Review the MSDS, to determine the protective equipment, spill clean-up, and disposal protocols that are necessary.
- ✓ Wear appropriate personal protective equipment (mask, safety glasses, lab coat, gloves and closed- toe shoes), and contain the spilled material first using an appropriate spill kit if required.
- ✓ In the event of a biological contamination, remove rotor from centrifuge and carefully retrieve unbroken tubes with plastic forceps if necessary. Wipe outside of rotor and inside the wells with disinfectant, and dispose of broken tubes in a sharps container.

    - ❖ **Disinfectant: a preparation of 1/10 dilution of bleach (Sodium hypochlorite), is effective in most situations but must be rinsed thoroughly with distilled water afterwards as it may cause discoloration of anodized surfaces.** Strong bases and/or high-pH solutions can damage aluminum rotors and components.

- ✓ After proper decontamination, carriers, rotors etc. can be washed with a mild detergent according to the manufacturer's instructions.
- ✓ If a spill has contaminated the centrifuge chamber, thoroughly wipe the inside of the chamber with disinfectant saturated towels. Allow for adequate contact time before wiping up excess liquid. Proceed to wipe out with 70% Ethanol.
- ✓ Report the spill to your supervisor, who will advise the user on the best way to clean up the spill in addition to documenting the clean-up procedure in the log book.
- ✓ If necessary, the rotor and all rotor components can be autoclaved at 121°C for up to one hour. Remove the lid from the rotor and place the rotor, lid, and O-ring in the autoclave upside down.

- ➢ Ethanol (70%)or hydrogen peroxide (6%) may be used on all rotor components, including those made of plastic. Use the minimum immersion time for each solution, per laboratory standards.
- ➢ While Beckman Coulter has tested these methods and found that they do not damage the rotor or components, no guarantee of sterility or disinfection is expressed or implied. When sterilization or disinfection is a concern, consult your laboratory safety officer regarding proper methods to use.

Ultra Centrifuge quick reference guide:

- Ensure that the rotor you select is class and speed rated for the Ultra or High Speed Centrifuge.
- Check centrifuge log book for any problems listed from previous runs. Log your run.
- Turn POWER switch ON.
- Pre-run the vacuum while you pre-cool the ultracentrifuge. Never leave the chamber open for significant periods when it is below ambient temperature.
- Select appropriate rotor and check physical conditions including cleanliness, O-rings (greased), lock screw threading and over speed disc.
- Balance your tubes and load up the rotor before placing it in the ultracentrifuge.
- Wipe off any condensation build-up.
- Install rotor in ultracentrifuge. Turn the rotor gently by hand to ensure it is seated properly on the spindle. Close the door.
- Start the vacuum.
- Reset OVERTEMP control to maximum allowable sample temperature only if essential, otherwise use the default setting.
- Set run parameters from the touch screen or control panel: TIME, TEMP and SPEED.
- Push START button when vacuum gets to the first stage.
- **Watch your ultracentrifuge as it accelerates**. Excessive vibration or noise as the speed builds up through 1500-3000 rpm may indicate a balance problem, especially with the swinging bucket rotor, and most incidents occur in this initial acceleration phase. Hit the stop button immediately at the first sign of a problem. Wait until the centrifuge has reached full speed before leaving the room.
- After the run is completed, press vacuum to vent the chamber.
- Remove the rotor from the ultracentrifuge and place aside on a bench top (not on centrifuge). Close chamber door.
- If any spillage occurred, the rotor and ultracentrifuge should be cleaned immediately.
- After every use and if no spills occurred, wipe out the inside of the ultracentrifuge with a sponge and distilled water, then wipe and return the rotor to storage upside down.
- Make a note of any problems in the logbook.
- Turn off the ultracentrifuge.
- After all runs are completed, run the vacuum with the temperature set to ambient to flush the vacuum system.
- Release the vacuum, turn off the POWER to the ultracentrifuge.