


STANDARD OPERATING PROCEDURE				
	Ultracentrifuge Operation at Ragon Institute		SOP#	RIO-SOP-003
	Originated by:	Alicja Trocha	Date:	23 November 17
	Laboratory:	Walker Laboratory	Pages:	1 of 2
	Approved by:	Alicja Trocha		

The Ultracentrifuge usage permission is granted upon completing individual training (each floor have designated group)

Upon receiving training you will get and individual PIN code which must be kept secret as entry of that code will be used to validate any PROBLEMS with the machine and group which user who sign up for the machine might be charged with repair costs.

I. PURPOSE:

The purpose of this procedure is to outline the procedure to be carried out as uniform training for all new Ragon employees


II. SAFETY:

This Training is implementing to ensure safety to all users as well as proper operation of the equipment.

III. REQUIREMENTS: Qualified trainer

IV. PROCEDURE:

1. Before operating any ultracentrifuge, contact the appropriate centrifuge manager (should be listed near the centrifuge). This manager will give you in-person training to operate the machine and a user ID/access code.
2. Ultracentrifuges can be used with interchangeable rotors. Be sure to familiarize yourself with the rotor you wish to use, and purchase appropriate ultracentrifuge tubes, such as Beckman Coulter centrifuge tubes (25 x 89 mm) (product number 326823) for the AH 629 rotor.
3. Sign up for an ULTRA in advance'
4. To operate the ultracentrifuge, type in your ID, press enter, type in your password, and press enter. For the machine to register inputs or selection, you need to press enter on the keypad after typing in/making your selections.
5. If you wish to cool the ultracentrifuge and rotor, begin cooling before starting sample preparation, because the cooling process can take an hour or more to reach 4C. To cool rotor buckets, place them in the 4C fridge before sample preparation. Cool the rotor in the ultracentrifuge. To place the rotor in the ultracentrifuge, lower the rotor directly down onto the center attachment so that it clicks in place. The rotor should be able to spin freely when inserted correctly. To cool the chamber, close the ultracentrifuge, set the temperature, and turns on the vacuum.
6. Prepare samples so that 36 total mL of fluid is in each tube. Do not under-fill the tubes, because they may collapse in during centrifugation, spilling your sample and making the tube difficult to remove. Over filled tubes risk spilling into the rotor bucket. From this point on, take care to handle buckets carefully to avoid spilling tube contents into the bucket.
7. In TC BL2+ Tightly screw on the caps to the rotor buckets, making sure that the correct cap is placed on the matching bucket. The number from each bucket should be aligned with the number on the bucket as closely as possible.
8. To remove samples from the biosafety hood, **disinfect all buckets** and the rack with D125 and then ethanol. The equipment must be sealed tightly and disinfected so that it safe to take into BSL-1 laboratory spaces.
9. Before taking samples to the ultracentrifuge, measure the mass of each rotor bucket. All buckets should have a mass within one gram of each other. If buckets are not within one gram of each other, return to biosafety hood and adjust contents appropriately. All buckets should be filled to reduce rotor strain. If you are not using all 6 tubes, fill the unused tubes with a similar density liquid (such as 20% sucrose layered with D10)
10. Weigh the tubes on closest scale. On 8th floor there are on Molecular Bench.
11. Place tubes in correct spot. Make sure to place samples in opposite cases (e.g. 1 and 4). The holder cap numbers should face outwards. The tube holders should be able to swing back and forth freely once correctly placed.
12. Remove the rotor from the ultracentrifuge chamber to carefully place each rotor bucket onto the rotor. As you place the bucket, check that the bucket location number, bucket cap number, and bucket number all match.

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Buckets should be securely fitted into the rotor. For a free swinging rotor, this means that they will not come out when you pull down on them and can swing freely (will rock back and forth without inhibition).

13. Carefully lower the rotor directly down onto the center attachment of the ultracentrifuge and check that it can spin freely. To start the spin, simply close the chamber, check that the parameters entered are correct, turn on the vacuum, and press start. It is easiest to enter parameters by touching the desired parameter on the screen, entering the value on the keypad, and pressing enter. Stay with the machine as it comes up to full speed. There should be no loud noises or signs of shaking as the machine come to speed. If any abnormal noises do occur, stop the machine and double check the balance and rotor set up.
14. Be sure to fill out the ultracentrifuge log which should be located near the machine, noting the starting number of revolutions and the spin parameters.
15. At the end of the cycle, release the vacuum to open the chamber and remove the rotor to its stand. Transfer the buckets to the biosafety hood in BL2+ TC room in sealed transport container lined with blue pad and open to retrieve samples. Remove the samples as soon as the cycle ends because the pellet will start re-dissolving if left for too long.
16. After taking out samples, carefully disinfect buckets, caps, and the stand with D125 for 15 minutes and then ethanol before returning all equipment to its original location.
17. Be sure to logout of the machine when done by pressing access, enter, logoff, and enter.

Safety Reminders:

1. Check maximum rotor speed before use. Never exceed allowable speed or else rotor may fail.
2. Be sure to measure the mass of all tubes before running samples. Tubes must be within one gram of one another. Find the closest available scale on the floor before you proceed with spin.
3. All buckets must be sealed tightly and disinfected with both D125 and ethanol before being removed from the biosafety hood.
4. Carefully attach buckets to the rotor. Buckets that cannot swing freely when necessary will unbalance the rotor. Loose buckets or an unbalanced rotor can destroy samples and damage the machine.
5. Ensure that all safety is in place when working with Ultracentrifuge as this machine operated wrongly can cause damage to the surrounding space and people.