

Protocol for Centrifuge Use

Warning: Centrifuges must be used correctly every time or you risk causing personal injury and/or thousands of dollars in damage to the centrifuges and rotors! If you are unsure about something, ask someone who has been trained!

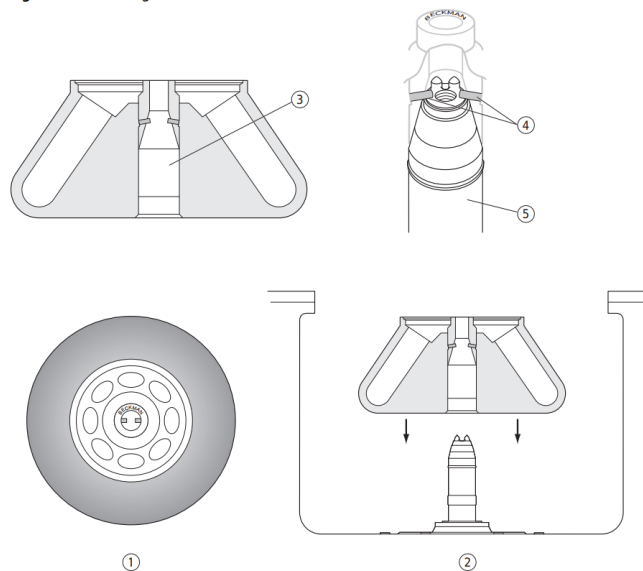
Preparing for your centrifuge run

1. Open centrifuge door and turn on.
2. Decide on the rotor you want to use. We have three:

Rotor	Max RCF (x g)	Common use	Tube volume
JLA-16.250	38 000	Pelleting cells (5000 x g)	~ 250 mL
JA-25.50	75 000	S30 spin (30 000 x g)	~ 40 mL
JLA-8.1000	15 000	Pelleting cells (5000 x g)	~ 1000 mL

3. Inspect the rotor that you want to use.
 - For the JLA-16.250 and JA-25.50, are there any obvious solids or liquids collected in the bottom of each hole where the tubes sit? If so, this must be cleaned with water, ethanol, and dried with a paper towel.
 - The same goes for the JLA-8.1000. Note that the JLA-8.1000 has carbon fibre inserts that must be placed within the rotor! Inspect the inserts to see if they have any solids or liquids in them (see step 6 for insert photo).
4. Place the rotor (without lid or inserts) inside the centrifuge carefully. Note that each rotor has pins in the centre that need to be in good condition and placed properly on the drive shaft. Do not use any rotors that have missing/broken pins!

Figure A.5 Checking the Rotor for Drive Pins



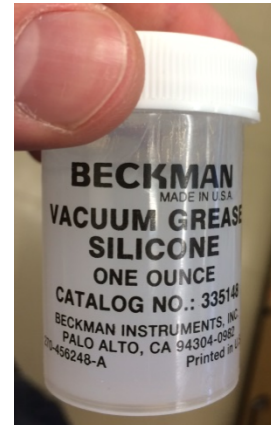
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5. Use a flashlight to look top down at the drive shaft to make sure the pins are placed correctly and aren't sitting directly on top of the drive shaft pins.
6. Place ALL of the carbon fibre inserts into the JLA 8.1000 rotor if this is the rotor you are using. Even if you are only spinning two buckets, you need to place all 6 inserts in. (The centrifuge tubes/buckets go within these inserts)



Sample preparation

7. Inspect the tubes you are planning to centrifuge your samples in for cracks, broken O-rings, missing O-rings, etc.
 - O-rings should be lubed up periodically with silicon lubricant (Beckman Vacuum Grease Silicone) found in the drawer below the fermenter. This can improve the seal and the lifetime of the O-rings. This can be done by removing the O-ring *carefully* with a pipette tip and applying lubricant with your gloves.



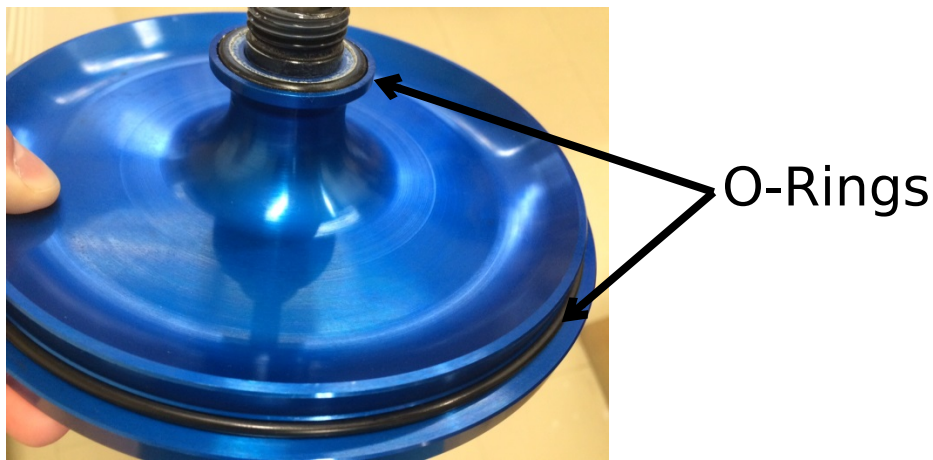
8. Your samples **MUST** occupy more than $\frac{1}{2}$ the centrifuge tube volume! Any less than this you run the risk of collapsing the centrifuge tubes and causing further issues.

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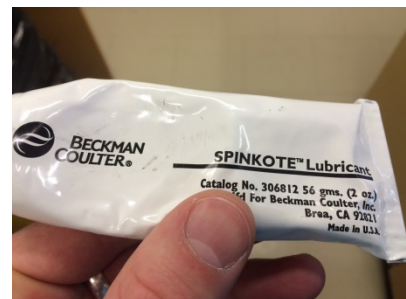
9. Centrifuge tubes **MUST** be balanced perfectly against another tube. Make sure you know what tubes are balanced with one another.
10. Once your tubes are balanced, they can be placed opposite of one another in the centrifuge rotor. If your tubes were sitting on ice, make sure to wipe the bottom of the tubes to remove any ice and liquids. Having ice in between the tube and the rotor during centrifugation can cause serious problems.

Sealing the rotors / lid placement

11. Inspect the rotor lids prior to centrifugation:
 - The JLA-16.250 and JA-25.50 lids have O-rings that need to be in good condition. Check these! You don't want your samples to be in a vacuum!



- All rotor lids have threads that need to be lubricated on a regular basis by **YOU!** Check to see if they are dry and apply a thin coat of Spin Kote.



12. Place the rotor lid on top of the correctly loaded rotor carefully and tighten to the centrifuge drive shaft. Don't be lazy and drop the lids in on an angle as this can damage the threads.

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13. There is a special way of tightening the JLA-16.250 and JA-25.50 rotor lids:
- Tighten the lower knob first to seal the rotor with the O-ring.
 - Tighten the upper knob second to attach the rotor to the centrifuge.
 - Perform in the reverse order when centrifugation is complete.



Upper Knob

Lower Knob

You are now ready to centrifuge!

14. Close the centrifuge lid and enter in the correct rotor, the RCF (x g), temperature (usually 4 °C), and time you would like to centrifuge. Double check these.
15. Start centrifugation by confirming the protocol by hitting enter (usually twice) and then start.
16. Do not leave the centrifuge until it has hit max speed and you know that it is balanced! Make sure to enter in your name into the centrifuge log book.

End of centrifugation

17. Open the centrifuge door and shut off the centrifuge. Remove the lid carefully and place it back where you obtained it.
18. Remove your centrifuge tubes carefully as to not disturb the pellet and place on ice.
19. Remove the inserts (JLA-8.1000) and rotors and check for any sediment and liquid where the tubes were placed. If there is any liquid or sediment, this needs to be cleaned ASAP with distilled water, ethanol, and then dried with paper towel. The JLA-16.250 and JA-25.50 rotors can be placed upside down on fresh paper towel on top of Styrofoam to allow for further drying.
20. Clean the centrifuge tubes ASAP, especially the 1 L tubes! We only have a limited number of these and other people need to use these.