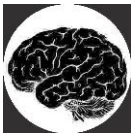
	SKILLS CENTER **STANDARD OPERATING PROCEDURE**	**A BIOFIZZ** **PRODUCTON**
Centrifuge 1 **Module Hours: 3.0**	**Effective Date: 01/17/2022** **PRQs** **Lab Safety** **Pipetting**	**Revision # 1.0** **M. Stowell** **Checked:** **Z. Hazlett-Klein, M. Guzie**

BACKGROUND

Various types of microcentrifuges used in the laboratory.

Centrifugation is a method that separates mixtures by applying a centrifugal force. A centrifuge is a motor driven device that puts a rotor in a rotational movement around a fixed axis. The rotational movment around a fixed axis creates a centrifigul force perpendicular to the rotation axis. A centrifuge works by enhancing sedimention under the influence of gravitational force (g-force). Under the influence of a g-force, substances separate according to their relative density. Many different types of centrifugal separation are utilized in molecular biology and include isopycnic, ultraf iltration, density gradient, phase separation, and simple pelleting to isolate dens materails. The microcentrifuge is most commonaly utilized for simple pelleting. In this method particles are concentrated as a pellet at the bottom of the centrifuge tube and separated from the remaining solution, called supernatant. More complicated methods of centrifugation are described in the centrifuge 2 module. Protocols for centrifugation typically specify the relative centrifugal force (rcf) and the degree of acceleration in multiples of g (g-force). Working with the rotational speed, such as revolutions per minute (rpm), is rather imprecise because the rcf is highly dependent upon the geometry of the centrifuge and rotor combination. Most microcentrifuges have a similar size and geometry and are primarilly used for simple pelleting procedures.

1. PURPOSE

	SKILLS CENTER STANDARD OPERATING PROCEDURE	**A BIOFIZZ**  **PRODUCTON**
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The purpose of this procedure is provide methods for the use of laboratory microcentrifuge for several types of pelleting procedures used in biological experiments.

2. SCOPE

This procedure applies to qualified skills center users.

3. RESPONSIBILITY

3.1. It is the responsibility of the user to understand and perform the procedure described in this document.
3.2. It is the responsibility of the user performing the procedure to fully document any deviations from the written procedure.
3.3. It is the responsibility of the user to become trained on the procedure. Only trained laboratory personnel can operate the instrument.
3.4. Personal protective equipment (PPE) including safety glasses or face shield and laboratory coat must be worn when using or handling centrifuge equipment.
3.5. Do not centrifuge samples together which can chemically react with each other when exposed to air.
3.6. Before starting the microcentrifuge, make sure that rotor is securely connected on the rotor nut.
3.7. Do not exceed the maximum rated speed of the rotor in use.
3.8. Make sure that filled containers are loaded symmetrically, by weight and by geometry, into the rotor to ensure the rotor is balanced.
3.9. Ensure that all microcentrifuge containers are capped and not overfilled, to prevent leakage.
3.10. Do not lift or move the microcentrifuge while the rotor is spinning.
3.11. Do not override the door interlock system while the rotor is spinning.
3.12. In the event of a power failure, do not attempt to retrieve the sample from the microcentrifuge until it has come to a full stop.
3.13. If a tube breaks or is damaged, remove the rotor and inspect the centrifuge and rotor for particles that remain in the rotor cavities or centrifuge and clean the rotor thoroughly.

3.14. If pathogenic, toxic, or radioactive samples are intended to be used in the microcentrifuge, it is the responsibility of the user to ensure that all necessary safety regulations, guidelines, precautions, and practices are adhered to accordingly.

4. DEFINITIONS

4.1. RCF – relative centrifugal force.
4.2. G – the force of gravity
4.3. RPM- revolutions per minute. The rate at which a centrifuge rotor is spun

5. MATERIALS/EQUIPMENT

5.1. Deionized water
5.2. Colored beads
5.3. 1.5 mL microcentrifuge tubes
5.4. Micropipettes
5.5. DI Water
5.6. Eppendorf 5280 microcentrifuge

6. MICROCENTRIFUGE OPERATION

6.1. With the power switch on, press the [open] button to open the chamber door.
6.2. Load the samples into the rotor. Always run the rotor with a balanced load. Close the chamber door firmly till you hear the lock mechanism click.
6.3. Preset run conditions (rcf/rpm, temperature, brake speed):
  6.3.1. Set run speed using the speed dial, selecting between rpm and rcf by pressing [Speed/rcf]. The set speed will be illuminated in the speed display.
  6.3.2. Set run duration using the time knob. Times between 1 and 60 minutes and hold can be selected. The set time (time remaining) will be illuminated in the time display.
  6.3.3. For a continuous run (hold), turn the time dial clockwise to the limit stop. Two minus signs (–) will be illuminated in the time display. The duration of a continuous run can be checked mid run by pressing [Start].

6.3.4. The brake intensity and acceleration can be changed by pressing [Fast/Slow]. If the green “fast” control lamp is lit, the unit accelerates and decelerates fast. If the green “slow” lamp is lit, the unit accelerates and decelerates very slowly.

6.4. Press [Start].

6.5. Changing run conditions during run (chamber door closed; optional):

6.5.1. Change run speed by holding [Preset] at the same time as adjusting the speed dial. The new run speed is set once [Preset] is released.

6.5.2. Change run duration by holding [Preset] at the same time as adjusting the time dial. The new run duration is set once [Preset] is released.

6.5.3. Change brake intensity and acceleration by pressing [Preset] at the same time as [Fast/Slow].

6.6. Allow the set time to count down to zero or end the run by pressing [Stop].

6.7. When the rotor stops spinning, press [open]. Unload the rotor.

6.8. For short centrifuge runs the [Quick] key may be used. The centrifuge starts and will remain running as long as the [Quick] button is pressed. The operating time will be indicated in seconds in the time display.

7. TROUBLESHOOTING

7.1. Beads cannot be pipetted easily

7.1.1. Cut the pipette tip open with razor/scissors

7.2. Microcentrifuge does not spin.

7.2.1. Check that the microcentrifuge is plugged in and turned on.

7.3. Microcentrifuge makes a rumbling noise and shakes when the run starts.

7.3.1. Stop the run immediately.

7.3.2. Either the rotor is unbalanced or the rotor is not screwed in properly.

7.3.3. Check each, correct as needed, and try run again.

7.4. Microcentrifuge makes a loud light unusual *whirring* noise during run

7.4.1. Stop the run immediately

7.4.2. Some microcentrifuges require lids for the rotor as well as a closed hood.

7.4.3. If applicable, make sure the rotor lid is firmly closed onto rotor.

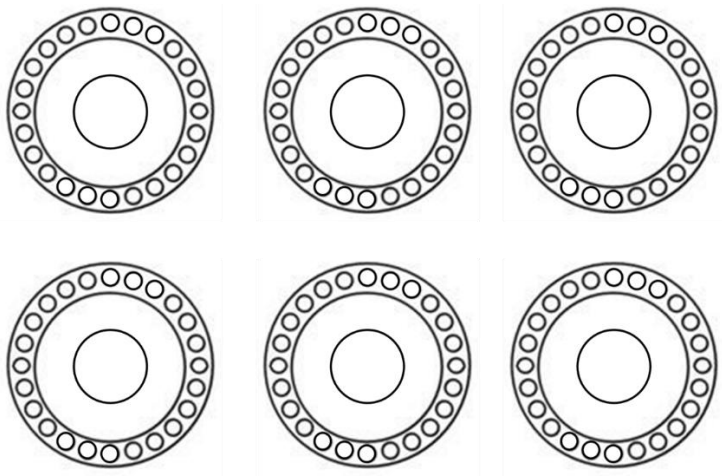
7.4.4. Close centrifuge hood and try again.

8. REFERENCES

9. MODULE METHODSTASK

This task will test your ability to properly load and operate the microcentrifuge and use the microcentrifuge for pelleting several different substances.

1.0 Balancing a centrifuge is one of the most critical aspects of proper centrifuge usage. This is even more critical at high rcf as the relative mass imbalance increases with rcf causing greater imbalance. Even though microcentrifuges operate at a relatively low rcf, proper balancing is important for the long-term operation of a microcentrifuge. Choose 3 even and 3 odd centrifuge tube numbers (for example 2, 6, 12 and 5, 9, 15) and diagram the proper loading of the microcentrifuge rotor using the rotor images below. For this case, all tubes are presumed to have equal weight/volume.



2.0 Pelleting of affinity agarose used in batch purification of His-tagged proteins is a common practice in protein purification. Here we will use two types of microspheres to test for optimal pelleting conditions of two different types of beads.

2.1 Pipette 500 μL of RED/BLUE Microspheres bead mixture (Phosphorex, 20 μm diameter (RED) and 1 μm diameter (blue)) into a 1.5 mL microcentrifuge tube.
- 2.1.1 Be certain to gently shake or vortex the bead mixture solution prior to pipetting
- 2.1.2 The mixture is stored as a slurry in the 4˚C fridge.

2.2 Make an appropriately balanced microcentrifuge tube

2.3 Test several speed (rcf) and time conditions for pelleting and report your results in the table below. Be sure to vortex the beads after every run so that they are adequately suspended.

2.4 Indicate a recommended time and speed to pellet both types of beads at the lowest speed within 5 minutes.

2.5 Indicate a time and speed to pellet mostly one bead type, indicate which one, but not the other.

2.6 Indicate if there are conditions that would allow you to pellet the blue beads and not the red beads.

2.7 When you are finished. Pipette the bead solution back into the bead mixture bottle for future use.

Sample/Run	Beads Mixture	DI Water	Total Volume
Test mixture			
Balance tube			

	Speed	Time	Result

Run 1			
Run 2			
Run 3			
Run 4			
Run 5			
Run 6			
Condition for pelleting both beads in 5 min			
Condition for mostly single bead pelleting			
Condition for mostly blue bead pelleting			