

Standard Operating Procedure (SOP)

This Standard Operating Procedure (SOP) describes basic chemical safety information for centrifugation. Prior to conducting work with a centrifuge personnel must obtain approval from their Principal Investigator (PI) and/or Supervisor and attend the appropriate laboratory safety training. The PI must complete the Lab-Specific Use Procedures section and provide their personnel with a copy of this SOP.

Print a copy of this SOP, sign it, and insert into your Safety on Site (SOS) Binder.

Centrifugation



Department:	
Date SOP was written:	
Date SOP was approved by PI/lab supervisor:	
Principal Investigator:	
Principal Investigator Signature:	
Internal Lab Safety Coordinator/Lab Manager:	
Lab Phone:	
Office Phone:	
Emergency Contact: <i>(Name and Phone Number)</i>	
Location(s) covered by this SOP: <i>(Building/Room Number)</i>	

Type of SOP: Process Hazardous Chemical Experiment Equipment Use

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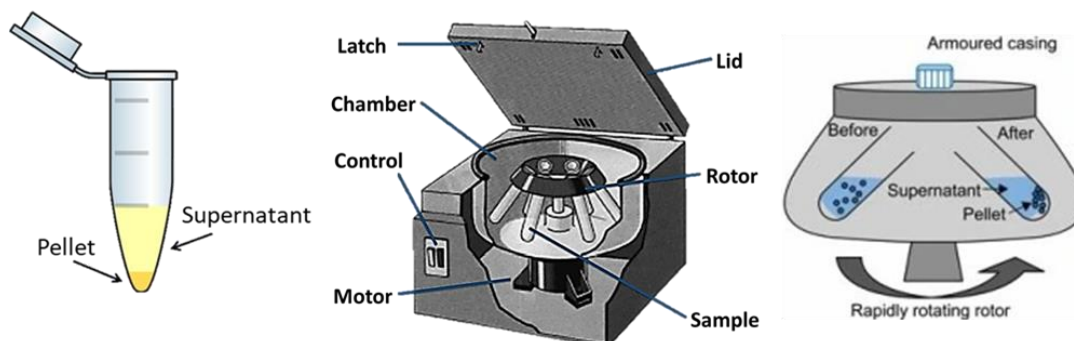
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Read and review any applicable manufacturer/vendor safety information before developing the standard operating procedures and performing work.

***** NOTE: Each section needs to be complete with clear and detailed information based on the blue/italic font instruction. SOP must be approved and dated by the PI or lab supervisor.**

1. Purpose and Scope of Work/Activity:

The process of centrifuging samples is extremely useful and employed in a variety of laboratories. A centrifuge spins samples at a high speed, the centrifugal force causes denser materials to travel to the bottom of the centrifuge tube rapidly. This allows for the separation of liquid and solid samples.



The information below addresses general laboratory safety concerns while setting up and operating a centrifuge. Modify this SOP with your laboratory's specific operational procedures with the help of the Principal Investigator and verify the SOP with EH&S approval prior to working with a centrifuge. Ensure that the laboratory specific procedures outlined in this SOP are followed by laboratory workers at all times. This document is not designed to substitute hands-on training and supervision by experienced laboratory personnel.

2. Responsibility

Identify the personnel that have primary roles in the SOP and describe how their responsibilities relate to this SOP. If necessary, include contact information.

Principle Investigator

- Must provide their laboratory with a copy of this SOP, must sign this SOP, and ensure that all members of their laboratory sign and understand this SOP.
- Must ensure that their laboratory personnel have obtained the appropriate general laboratory safety training, laboratory specific training, and any refresher training.
- Must ensure that trained personnel conduct repairs and maintenance on the centrifuge as needed.

Laboratory Personnel











- Must obtain all necessary laboratory safety training, refresher training, and laboratory specific training.
- Must read, sign, understand, and follow this SOP when using the centrifuge.
- Must read the safety data sheet (SDS) for every compound that is being centrifuged.

3. Specific Hazards and Controls

3.1. Chemical Hazards

The chemical hazards vary depending on the experiment. Samples may be toxic, flammable, carcinogenic, pyrophoric, etc. All the necessary precautions for these compounds should be taken into account when conducting work with a centrifuge. Read the SDS for the compounds being used with a centrifuge.

List all of the specific chemicals typically used and their hazards.

Chemical/Material (Name, Cas #, other ID)	Hazards	GHS Symbol		
		Health Hazard 	Flame 	Exclamation Mark 
		Gas Cyliner 	Corrosion 	Exploding Bomb 
		Flame Over Circle 	Environment 	Skull and Crossbones 
<p><i>Example:</i> Tetrahydrofuran (109-99-9)</p>	<ul style="list-style-type: none"> - highly flammable liquid and vapor - harmful if swallowed - causes serious eye irritation - may cause respiratory irritation - suspected of causing cancer 			

3.2. Biological Hazards.

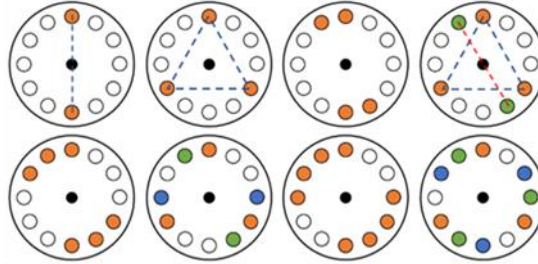
Biological hazards vary depending on the biological agent and materials used. Centrifugation creates aerosols that can increase the risk of exposure to a hazardous agent or materials. Read the PSDS for the agents being used with a centrifuge.

Biological Agent (Name, ID)	Hazards
<p><i>Example:</i> Herpes Simplex virus</p>	<ul style="list-style-type: none"> - falls under aerosol transmissible disease/pathogens - transmitted via aerosols - may cause associated disease

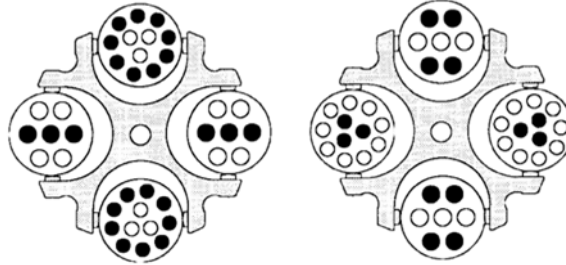
3.3. Hazards and Controls

In general the main hazards and controls associated with centrifugation are:

Risks and Hazards	Controls
<p>Mechanical failure or strain Centrifuge explosion Uncontrolled movement</p>	<ul style="list-style-type: none"> • Properly balance the centrifuge before using it. Examples of some appropriate methods are shown below. Centrifuged are most easily balanced with container pairs that are placed directly across from each other.



- For buckets and adapters, load all of the buckets and adapters even if you are only using two out of four. Ensure that they are properly balanced.



- Ensure that the centrifuge tubes are balanced by mass not by volume, always weight your tubes with the caps on (if you are capping the samples in the centrifuge) to ensure that they are balanced before centrifuging.
- The faster a centrifuge spins the more small differences in weight have an effect and lead to possible mechanical failure and centrifuge explosion. Therefore ultracentrifuges must be carefully balanced by weight since they run at extremely fast speeds.
- Never leave the centrifuge unattended until it is at the desired operational speed. Wait until the centrifuge gets to speed and observe if there are any signs of mechanical failure (e.g. shaking, odd noises). This type of failure usually occurs upon start up so it is imperative that you observe the instrument during that time.
- If the centrifuge starts to wobble, shake, or make an odd noise turn the centrifuge off immediately. Centrifuges will often vibrate when working, however if the centrifuge is wobbling or shaking something is wrong. Typically this is caused by user error (e.g. not balancing the centrifuge properly), but it can also indicate mechanical failure.
- If mechanical failure of the centrifuge occurs, immediately turn the centrifuge off and unplug the power cable. Post a sign on the centrifuge to inform lab members that the machine is not working properly. Wait for a qualified service technician to inspect and repair the machine before using it.
- Make sure that the rotor is in good working condition before using the centrifuge. Do not switch rotors, each centrifuge is equipped to handle a specific type of rotor and changing the rotor can led to mechanical failure.
- Always use a centrifuge on a level and firm surface, never use a centrifuge on an uneven or slanted surface.
- The centrifuge should be secured to the bench top by suction cups, wheel brakes, or another method.
- Always follow the operational instructions for the specific centrifuge that is being used. Never circumvent the safety features of the centrifuge.

	<p>Make sure that all parts of the centrifuge are in working condition prior to use.</p> <ul style="list-style-type: none"> • Always wear the appropriate personal protective equipment.
Pinch hazard Loose articles caught	<ul style="list-style-type: none"> • Secure loose hair and clothing. • Never open a centrifuge when it is running. • Never touch a moving centrifuge, this can lead to severe injury or damage to the centrifuge. • Allow the centrifuge to come to a natural stop, do not try to slow or stop a moving centrifuge.
Broken glassware or material	<ul style="list-style-type: none"> • Do not place a centrifuge on a shelf where the normal operating vibration of the centrifuge will cause reagents or materials to fall off. • Inspect all tubes and containers for cracks before using them. Never use a centrifuge tube that is cracked. Broken centrifuge tubes can lead to imbalance of the machine causing failure and injury. • Always use correct well-fitting tubes for each centrifuge.
Chemical hazards	<ul style="list-style-type: none"> • Always wear the appropriate personal protective equipment. • When centrifuging hazardous chemicals, use the centrifuge in a fume hood or glovebox. If the centrifuge cannot be used in a fume hood, the centrifuge tubes should have airtight caps and only be filled/opened in the fume hood. • Always use safety centrifuge cups to contain any potential spills and prevent aerosols. • Wait 10 minutes after the centrifuge has stopped before opening the lid to allow any aerosols to settle before opening the lid. At a minimum never open the centrifuge lid until it has come to a complete stop. • Never overfill centrifuge tubes or containers. This can cause the solution to splatter when it is being centrifuged.
Biological hazards	<ul style="list-style-type: none"> • If centrifuging materials from a Biosafety Level 2 or higher, rotors must have aerosol containment including sealed/gasketed lid or safety caps for the bucket/adaptor or be used inside a biosafety cabinet. • The rotor with the lid or the bucket/adaptor with safety caps must be taken inside the BSC. Centrifuge tubes must be loaded and unloaded inside a biosafety cabinet, and the outside of the rotor or the bucket/adaptor with safety caps should be wiped with disinfectant. • Wait 10 minutes after the rotor comes to a complete stop before opening the lid.
Radiological hazards	<ul style="list-style-type: none"> • Centrifuges used for radioactive material should be labeled with yellow and magenta tap or labels depicting ‘Caution - Radioactive Material.’ • If centrifuging radioactive materials, the centrifuge and tubes must be kept behind an appropriate shield for the material you are using. • Use only centrifuge tubes with screw caps and “o” rings and ensure the caps are on tight. • Perform a contamination survey after each centrifuge run to ensure no radiological contamination is present. If contamination is present, decontaminate the centrifuge. Contact the Radiation Safety Division for guidance. Do not allow other lab personnel to use a radiologically contaminated centrifuge.

4. Hazard Control(s)

4.1. Engineering/Ventilation Controls

Centrifuges, based on the materials, should be located in a fume hood, glovebox, biosafety cabinet, or other engineering control to prevent exposure (e.g. inhalation).

If the centrifuge cannot be located in the appropriate engineering control and inhalation hazards or aerosol hazards are present, load the tubes within the appropriate engineering control and use an aerosol-tight centrifugation with aerosol-tight centrifuge tube caps.

4.2. Administrative Controls

Documentation

- The centrifugation standard operating procedure (SOP) must be completed and signed by every member of the laboratory.
- Safety data sheets (SDS) for each material should be reviewed prior to use.
- Manufacturer operating manual should be reviewed prior to use.

Training

- Training must be completed prior to working in the laboratory.
- Process specific training must be completed prior to working with a centrifugation.
- Basic mandatory trainings include laboratory safety fundamentals, hazardous waste management, and hazardous materials incidents emergency procedures.

4.3. Personal Protective Equipment

EYE PROTECTION: ANSI Z87.1-compliant safety glasses or safety goggles if a splash hazard is present.

PROTECTIVE CLOTHING: Long pants (or equivalent) completely covering legs, closed toed shoes, and a traditional lab coat or flame resistant Nomex® lab coat when working with flammables.

HAND PROTECTION: Nitrile or neoprene gloves are typically adequate for minor splashes. Thicker gloves should be used for longer operations, larger quantities, or direct contact. Consult the SDS and/or your PI to determine whether the material or process requires alternative hand protection.

5. Location of Nearest Emergency Safety Equipment

ITEMS	LOCATION
EYEWASH/SAFETY SHOWER	
CHEMICAL SPILL KIT	
FIRST AID KIT	
FIRE EXTINGUISHER	
TELEPHONE	
FIRE ALARM MANUAL PULL STATION	
OTHERS/DETAILS:	

6. Step-by-Step Operating Procedure

Provide the steps required to perform this procedure.

For a process: Write enough detailed description of the procedure to guide the user through the process including details of startup, normal condition operation, temporary operation condition, and emergency shut-down, etc.

Also cover enough information, such as:

- 1. Chemical concentrations, gas amount*
- 2. Pressure limits, temperature ranges*
- 3. Flow rates*
- 4. Special safety equipment is to be utilized.*
- 5. Schematics or pictures for complex setups.*
- 6. Highlight safety precautions put in place*
- 7. What to do when an upset condition occurs*
- 8. What alarms and instruments are pertinent if an upset condition occurs*
- 9. If lockout/tagout is required*

Some tips:

- Use numbers for steps and sub-steps that have to be performed in a specific sequence*
- Use bullets for steps or items that can be performed in any order.*
- Solicit ideas for other users.*
- Include a flow diagram to help interpret more complex procedures.*
- Include pictures and label different components.*

For Equipment: Describe the step-by-step procedure for using the equipment properly. Include details for potential equipment failure if something is done improperly in the procedure. Describe how to power down the equipment at the end of use.

BASIC OPERATING PROCEDURE

You must edit this procedure to reflect your laboratory's specific procedure. The procedure below is meant to offer a general procedure for guidance and safety considerations when using a centrifuge.

1. Inspect the centrifuge to ensure it is working properly, there is no damage, and that the centrifuge is able to move freely.
2. Select the appropriate centrifuge tubes or containers, inspect them to make sure there are no cracks or flaws. Discard any tubes/containers that are cracked or flawed.
3. Fill tubes with the desired liquid.
 - a. Never overfill or under fill the tubes or containers (follow the manufacturer's limits).
4. Make sure that the centrifuge tubes are balanced, use a balance to ensure that the weights of both tubes match.
 - a. Do not balance based purely on volume! This is especially true for solutions with different samples or different concentrations of the same sample.
5. Tightly secure the lids on the centrifuge tubes.
6. Make sure that the outside of the centrifuge tubes are clean and dry before placing them in the centrifuge.

7. Balance the tubes within the centrifuge.
 - a. Pictures of properly balanced centrifuges are shown above (Section 3.2. Hazards and Controls). Tubes should be weighted to ensure that they balance properly.
8. Close the lid. Make sure the lid has a tight fit.
9. Set the run speed and run time. (Never use the rotor's maximum run speed).
10. Do not leave the centrifuge until it is operating at full speed and the machine seems to be running smoothly. Ensure that there is no abnormal noise or vibration.
11. If you notice an unusual noise or vigorous shaking, immediately turn the centrifuge off and remove it from the power source.
 - a. Typically this is caused by the centrifuge not being balanced properly. If the problem does not resolve and the centrifuge is balanced properly, most likely the centrifuge is broken, do not use the centrifuge until it is fixed.
12. Once the centrifuge has completed its run, allow the centrifuge to stop its rotation before opening the lid. Never open the lid or touch the centrifuge before it has stopped its rotation. **NEVER TRY TO HASTEN THE STOPPING PROCESS!** Stopping the machine prematurely can lead to injury and mechanical failure.
13. Preferably do not open the lid for at least 10 minutes after the rotation has stopped to allow the aerosols that were released during centrifugation to settle.
 - a. If centrifuging hazardous materials use the centrifuge in a fume hood or glovebox.
 - b. If centrifuging biosafety level 2 materials the centrifuge must be used in a biosafety cabinet or rotors must have aerosol containment.
 - c. If centrifuging radioactive materials, use the appropriate shielding during this process.
14. After the centrifuge has completely stopped moving, you may remove your samples from the centrifuge.

7. Special Handling Procedures, Transport, and Storage Requirements

Describe special handling and storage requirements for hazardous chemicals/gases in the laboratory, especially for highly reactive, unstable and highly flammable materials and corrosives. Describe transport and secondary containment requirement, between the laboratories or between facilities.

8. Preventive Maintenance

Clean up and preventive maintenance is important for keeping equipment in safe working order. Any regular maintenance and/or calibration frequency for research equipment, instrumentation and/or facilities should be included here.

A white paper describing the proper routine maintenance of centrifuges was published by Eppendorf, https://handling-solutions.eppendorf.com/fileadmin/Community/Sample_Handling/Maintenance/White-Paper_014_Centrifuges_Routine-Maintenance- eng.pdf.

- Some high-speed centrifuges (e.g. Ultra-centrifuges) must be certified annually, know the certification schedule for the centrifuge you are using and ensure it is certified according to the manufacturer's recommendation.
- Always follow the manufacturer's instructions for cleaning and maintaining the centrifuge.
- Clean the centrifuge regularly. Moisture, chemicals, strong cleaning agents, and other materials can promote corrosion and lead to centrifuge failure. Ensure that the centrifuge is kept clean and dry.
- Decontaminate the rotor after using biological materials, to clean the rotors and cups use 10% bleach for 30 minutes followed by 70% ethanol and let air dry.
- Inspect the rotor regularly, do not use a centrifuge if the rotor shows any defects or corrosion.
- Never use abrasive wire brushes to clean the rotors or any associated parts of the centrifuge.

- Cleanup any spills immediately.
- Ensure that your centrifuge is properly lubricated. (If necessary lubricate the bucket grooves, pivots of swing-bucket rotors, and rubber seals.)
- Check the seals of aerosol tight lids/caps, replace as needed.

9. Monitoring and Safety Systems

This section includes a list of all monitoring systems such as gas detectors, safety interlocks, equipment guards, fail safe control logic, etc. noted.

10. Waste Disposal/Cleanup

Dispose of any hazardous waste generated through UCI EH&S. A hazardous waste pick up can be scheduled by completing a Hazardous Waste Pick Up Form, via the internet, <https://www.ehs.uci.edu/programs/enviro/> or texting hwp@uci.edu (detailed instructions can be found at <https://www.ehs.uci.edu/apps/waste/Text%20a%20Chemical%20Waste%20Request.pdf> or https://www.ehs.uci.edu/apps/waste/text_to_collect.jsp).

11. Emergency Response Plan

In this section describe any special procedures for spills, releases, or fires. Indicate how accidental events should be handled and by whom. List emergency contact numbers.

11.1. First Aid Procedure

If inhaled

1. Move to fresh air
2. Have victim rest in half-upright position
3. Seek medical attention immediately

In case of skin or eye contact

1. Immediately flush skin or eyes (eyewash station) with plenty of water for at least 15 minutes
2. Remove contaminated clothing and shoes
3. Get medical attention immediately

If swallowed

1. Do not induce vomiting unless directed to do so by medical personnel and never give anything by mouth to an unconscious person
2. Loosen tight clothing such as a collar, tie, belt, or waistband
3. Get medical attention immediately

11.2. Life-threatening emergencies

(Such as: fire, explosion, large-scale spill or release, compressed gas leak, valve failure, etc)

1. Evacuate the room and close the door behind you
2. Secure the room to prevent entry
3. Alert people in the area and activate the local alarm systems
4. **Call 911 – Tell the dispatcher the name of the gas or chemical.**
5. Provide local notification
6. Report to EH&S at x4-6200 within 8 hours
7. Complete online incident report at <https://www.ehs.uci.edu/apps/hr/index.jsp>

Identify the area management staff that must be contacted and include their work and home numbers. This must include the PI and may include the safety coordinator and facility manager.

11.3. In case personnel are exposed or injured

1. Remove the victim from the area if it is safe to do so
2. Follow first aid protocol as mentioned above
3. Provide safety data sheets (SDSs) for all chemicals to Emergency Medical Technician (EMT) or to the hospital
4. Report to EH&S x4-6200
5. Complete the online incident form <https://www.ehs.uci.edu/apps/hr/index.jsp> or Human Resources, Workers Compensation at x4-9152

Non-life threatening emergencies

1. Notify your supervisor or faculty staff
2. Report to EH&S x4-6200

Identify the area management staff that must be contacted and include their work and home numbers. This must include the PI and may include the safety coordinator and facility manager.

11.4. For spill & accident procedure

In the event of a small spill or release that can be cleaned by a trained local personnel follow below steps:

1. Use appropriate personal protective equipment and clean up material for chemical spilled
2. Double bag spill waste in the appropriate bags (e.g. red biohazard bags for biohazard waste), label and schedule a chemical waste pick-up

In case of large spill or release:

1. Evacuate the spill area
2. Post someone or mark-off hazardous area with tape and warning signs
3. **Call 911** and EH&S at x4-6200 for assistance
4. Keep the fire extinguisher nearby

Note: Fire extinguishers containing water are not suitable for flammable liquid fires

12. References

This section should include the references that were used to produce this SOP.

- Online SDSs can be accessed at <http://www.ehs.uci.edu/msds.html>
- Samira Schroeder, Eppendorf AG, Hamburg, Germany. Routine Maintenance of Centrifuges. Cleaning, Maintenance and Disinfection of Centrifuges, Rotors and Adapters. White Paper No. 14. https://handling-solutions.eppendorf.com/fileadmin/Community/Sample_Handling/Maintenance/White-Paper_014_Centrifuges_Routine-Maintenance-_eng.pdf
- Stanford Environmental Health & Safety. Centrifuge Safety. <https://ehs.stanford.edu/reference/centrifuge-safety>
- OSHA Quick Facts. Laboratory Safety. Centrifuges. <https://www.osha.gov/Publications/Laboratory/OSHAquickfacts-lab-safety-centrifuges.pdf>
- OSU Environmental Health & Safety. Safety Instruction. Centrifuge Safety. https://ehs.oregonstate.edu/sites/ehs.oregonstate.edu/files/pdf/si/centrifuge_safety_si080.pdf
- Centrifuge Maintenance. <https://www.youtube.com/watch?v=E0Kt79N9Sm4>.

13. Additional Notes and Attachments

In this section list any notes or attachments needed to implement this SOP.

14. Documentation of Training

- Any deviation from this SOP requires approval from PI.
- Prior to conducting any work with the equipment, designated personnel must provide training to his/her laboratory personnel specific to the hazards and procedures involved in working with this process.
- The Principal Investigator must provide his/her laboratory personnel with a copy of this SOP and a copies of any SDS provided by the manufacturer for any chemicals used.
- The Principal Investigator must ensure that his/her laboratory personnel have attended appropriate laboratory safety training or refresher training annually.

