

Phenol-Chloroform Extraction SOP

Summary

- Phenol-chloroform extractions are used to separate nucleic acids from proteins and lipids.
- Aqueous samples, lysed cells, or homogeneous tissue samples are mixed with a phenol-chloroform solution to extract the nucleic acids.
- Phenol is readily absorbed through the skin and causes burns, so precautions should be taken while handling the solutions.
- Chloroform enhances the ability of phenol to be absorbed dermally, so extra precaution should be taken in these procedures.
- Always work in a hood when handling these solutions.
- Always wear a lab coat, gloves and eye protection when handling phenol or solutions containing it.

What are phenol-chloroform extractions?

Phenol-chloroform extractions are used to separate nucleic acids from other cellular substances. A mixture of phenol, chloroform, and isoamyl alcohol is added to tissue samples to promote the partitioning of lipids and debris into an organic phase, leaving the DNA in the aqueous phase. The aqueous phase containing the DNA can be transferred to a clean tube after centrifugation for isolation of the DNA by precipitation.

Commercial solutions containing phenol and other materials are available for purchase as well. One popular brand is TRIzol® from Fisher Scientific that can be used to isolate DNA, RNA, and proteins from the same sample.

What are the hazards?



Chloroform is classified as toxic, a health hazard, and an irritant. Chloroform can be absorbed through the skin, and it can cause severe eye and skin irritation or be harmful if ingested. It is also classified as a possible human carcinogen.

Phenol is classified as toxic, corrosive, a health hazard, and environmentally damaging. Phenol can cause serious eye burns and skin damage, as well as pose a general risk to aquatic life and environments. It causes very painful burns that can bleach the skin and take a long time to heal. Phenol is a solid that

readily deliquesces, which can lead those not familiar with the material to assume droplets are simply water, leading to accidental burns or improper clean up.

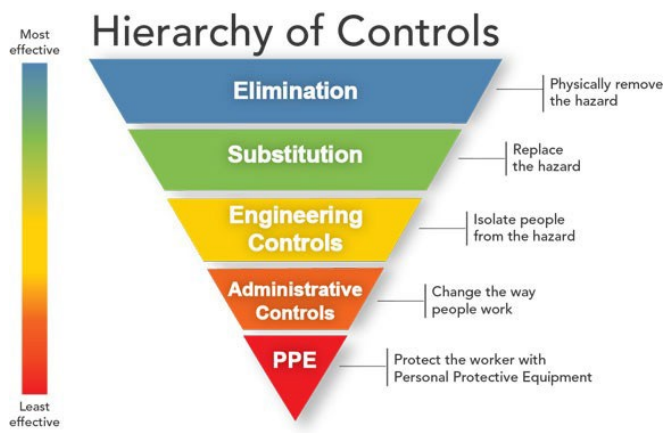
What activities could pose a risk?

If not using the proper engineering controls (i.e., a fume hood), exposure via inhalation can occur. Fume hoods also afford protection from splashes as the sash creates a barrier when used at the appropriate working height. Handling of tubes should be minimized when working with these chemicals; consider leaving tubes in racks when actively adding chemicals to minimize potential spills and exposure. Ensure liquid is not present on caps of microfuge tubes when opening to avoid accidental splashes. When working with these extractions, centrifugation is necessary. This can pose a risk of spilling and dropping of samples in transport, therefore, the samples should be in tightly secured centrifuge tubes before moving in a rack. Ensure proper centrifuge technique is being used in these procedures (i.e. lid secured and properly balanced). Phenol and chloroform have various chemical incompatibilities which should be considered when storing these solutions as well as handling waste. This list includes: inorganic acids, oxidizers, alkali metals, acetone/base mixtures, strong bases, and other less common chemicals. Phenol and chloroform should be stored in dark places.

How can exposures be minimized?

As with any other hazardous material, always conduct a thorough risk assessment and employ the hierarchy of controls to minimize risk when working with materials containing phenol and chloroform. Some specific applications of the hierarchy of controls to these hazards are listed below.

Always apply the controls in the order of most effective to least effective (see graphic), and apply as many controls as possible to reduce the risk to the lowest achievable level.



Performing these extractions pose a risk, but that risk can be mitigated using proper techniques following the procedures listed below.

Elimination/Substitution

- Only use phenol-chloroform extractions when other, safer alternatives for separation (i.e., salting out) are not possible.
- Work on as small of a scale as possible to minimize risks when handling these chemicals, providing a safer means of extraction.

Engineering Controls

- Always work in a chemical fume hood when working with, transferring, or opening bottles and tubes containing phenol and chloroform. Properly seal tubes and bottles before transport to minimize fume dispersal and splashing.

- Check that chemical fume hoods are operating at the proper face velocity before each use. Report any issues with fume hoods to the Solutions Center: <https://www.umass.edu/af-forms/physicalplant/service-request>.

Administrative Controls

- Consult the manufacturer's Safety Data Sheet and additional chemical information at <https://cems.unh.edu/umass/CEMS/SearchSDS>
- Locate nearest eyewash and shower and confirm that they are accessible and within 50 ft. of the work area.
- Check centrifuges and tubes for any defects before using them for procedures.
- Check microfuge tubes for liquid around the cap area before opening to avoid accidental splashing.
- Dispose or clean pipet tips and other materials used to contain or dispense solutions immediately to avoid contamination of surfaces or other potential sources of accidental exposure. For grossly contaminated items that are not cleaned, please dispose of those items as hazardous waste.
- Wipe down work surfaces after completion of work to avoid potential for accidental exposure. Alternatively, use bench paper to line the area and contain spills. Change bench paper frequently and when contaminated.
- Always wash your hands after handling solutions containing phenol.

Personal Protective Equipment

- Always wear appropriate eye protection consisting of safety glasses at a minimum. Depending on the quantity of solution or specific procedure being used, chemical splash goggles and potentially a face shield may be appropriate.
- Wear appropriate attire consisting of long pants, closed toed shoes, and an appropriately fitted lab coat. Lab coats should extend to the gloves to ensure there are no areas of exposed skin. If exposure to the lab coat occurs with solutions containing phenol, remove the coat immediately. Ensure the coat is laundered through the [lab coat program](#) prior to using again.
- Nitrile gloves must be worn at all times when handling solutions containing phenol or chloroform. Change contaminated gloves immediately. Double-gloving is recommended for ease of changing contaminated gloves and to afford additional protection.

Waste Handling

Treat all used solutions as hazardous waste. Since the biological component to these solutions have already been inactivated by the extraction process, it is not necessary to bleach these solutions (in fact, this can be harmful). Keep in mind chemical compatibility when storing waste. To have the waste picked up by EH&S staff, complete a Hazardous Materials Pickup Request form in [CEMS](#).

Exposure and Spill Procedures

In the event of a spill involving phenol or chloroform contained in a hood, it should be safe for lab personnel to clean up following the general procedure for small spills detailed in the [University's Chemical Hygiene Plan](#). People nearby should be alerted to the spill. PPE should be worn while cleaning

the spill. Apply paper towels or other appropriate spill absorbent material and place in a sealed container for disposal. For larger spills or spills outside the fume hood, the area should be evacuated (with the emergency exhaust/purge button pushed if safe and present) and EH&S should be immediately called (413-545-2682). If you ever feel unsafe cleaning up a small spill, please contact EH&S.

Exposures to hazardous materials should follow the general procedures for exposures outlined in the University's Chemical Hygiene Plan.

For an exposure requiring the use of a drench shower or eye wash:

- Have someone call 911 (report the building name, room number, and street address, located on the Emergency Action Plan located near the door to the lab) or 413- 545-3111 (or simply 5-3111 from a campus line) to report the incident and request medical help. If possible, communicate to the first responders the nature of the exposure.
- Help the affected individual to position their head over the eyewash and activate it, or position them under the drench shower and activate it as appropriate.
 - Always ensure your own safety before helping others. Only help if it is safe for you to do so.
 - Wear gloves, safety glasses, and a lab coat.
- If using an eyewash: Instruct the affected individual to open their eyes and roll them around while the water is flowing. Help them to hold their eyes open if necessary and safe to do so.
- If using a drench shower: Remove all clothing from the affected area while under the shower.
- Flush the affected area for 15 minutes with water.

For minor exposures such as a spill to readily accessible extremities (e.g. hand)

- Flush the affected area in a sink equipped with preferably potable water for at least 15 minutes.
- Go to University Health Services (UHS) for medical evaluation, and tell them you have had a lab exposure.
- Notify EH&S (413-545-2682) as soon as possible and complete the lab incident form (<https://ehs.umass.edu/lab-incidents-and-lab-incident-report-form>).

References

SDS for phenol and chloroform available through CEMS
(<https://cems.unh.edu/umass/CEMS/Dashboard>)

H.E. McKiernan, P.B. Danielson, *Molecular Diagnostics*, 3, 371-394, 2017.

[Harvard EH&S Phenol-Chloroform Extraction SOP](#)

[Fisher Scientific Phenol-Chloroform Extraction Procedure](#)

[Fisher Scientific TRIzol Instructions](#)