

STANDARD OPERATING PROCEDURE:
PHENOL:CHLOROFORM EXTRACTION (with TRIZOL[®])

PI: _____	Room & Building: _____
Department: _____	Research Group: _____
Date: _____	Pertains to Lab Protocol: _____

PROCEDURE

Attach the experimental protocol(s) for phenol:chloroform extractions with Trizol[®].

MATERIALS & HAZARDS

Principal Materials Used	Corrosive	Irritant	Sensitizer	Reproductive toxin	Acutely Toxic	Carcinogen	Flammable	Combustible	Other Comments:
Phenol	X	X						X	See comment 1, below.
Chloroform		X		X		X			See comment 2, below.
Isoamyl alcohol		X						X	
Trizol [®] (30-60% phenol + 30% Thiocyanate compound)	X	X			X			X	See comment 3, below.
Isopropanol		X					X		
DEPC-treated water									

Other comments:

1. Phenol is readily absorbed through the skin and can cause severe burns to the eyes and skin. Rinse contacted areas with a large volume of water and wash with soap and water. Systemic effects include damage to the liver and kidneys.
2. Adding chloroform to phenol enhances the ability of phenol to be absorbed by the skin. Systemic effects include damage to the liver and CNS.
3. Trizol is corrosive to the eyes and may cause severe damage including blindness. Causes severe burns to eyes, nose, skin, throat, lungs, and stomach. It is toxic by inhalation or if swallowed. May damage CNS, kidneys, liver, and pancreas.

ENGINEERING/VENTILATION CONTROLS

All procedures involving the transferal of phenol, chloroform or Trizol[®] should be performed in a chemical fume hood.

RECOMMENDED PERSONAL PROTECTIVE EQUIPMENT

The level of skin and eye protection should be selected based on the potential for splashing and other forms of exposure.

Minimum potential for splash & exposure:

- ✓ Chemical splash goggles
- ✓ Single pair of neoprene rubber gloves recommended (however, may be bulky)
 - Double nitrile gloves acceptable, but note that there is a shorter breakthrough time.
 - Double-gloving is recommended, given the diversity of chemical hazards and highly corrosive chemicals involved. Consider a different color and chemistry with each layer.
 - Immediately replace with new gloves when splash occurs.
- ✓ Protective clothing (e.g. impervious lab coat, sleeves, closed-toed footwear)

When using or transferring large quantities, or for spill clean-up:

- ✓ Chemical splash goggles
- ✓ Heavier gloves made of Viton™, or Silver Shield™, or double neoprene rubber gloves
 - Double-gloving is recommended, given the diversity of chemical hazards and highly corrosive chemicals involved. Each layer may be a different color and chemistry.
 - Immediately replace with new gloves when splash occurs.
- ✓ Chemical resistant, impervious apron/smock/lab coat (PE, PVC, natural rubber, neoprene, or Silver Shield™) that ties from behind.
 - Avoid using the traditional cotton-polyester white lab coat, which readily collects/absorbs compounds.
- ✓ Protective clothing (e.g. sleeves, impervious boots or PVC disposable shoe coverings, closed-toed footwear)

ADDITIONAL PRECAUTIONS

- When centrifuging material:
 - Use plastic bottles and tubes instead of glass to reduce the risk of breakage.
 - **Note:** Chloroform and phenol are capable of degrading some types of plastics. Check the chemical resistance or compatibility of materials selected prior to use. FEP/PTFE/PFA (Teflon fluorinated ethylene propylene/polytetrafluoroethylene/perfluoroalkoxy) is recommended.
 - Plan to use aerosol-proof rotors or safety caps during centrifugation to ensure aerosol containment, knowing a centrifuge tube may break while spinning.
 - Wait at least 10 minutes before opening the centrifuge to allow aerosol(s) to settle.
- Keep liquid phenol tightly closed and away from heat and light. Store away from inorganic acids and oxidizers (such as chlorine, bromine, and calcium hypochlorite).
- Chloroform reacts violently with alkali metals, such as potassium and sodium, a mixture of acetone and base, a strong base (such as potassium and sodium hydroxide, potassium *t*-butoxide, sodium methoxide, and sodium hydride). It reacts explosively with fluorine and dinitrogen tetroxide. In the presence of light, chloroform undergoes autoxidation to generate phosgene; this can be minimized by storing chloroform in the dark, under nitrogen. Do not store in aluminum containers.

MATERIAL SAFETY DATA SHEETS

MSDSs are available electronically via EHS Department's Web page:

<http://www.uos.harvard.edu/ehs/msds/>. An option, but consider collecting in a binder the MSDSs that arrive with each order.

WASTE DISPOSAL

Refer to the *Laboratory Waste Guide* posted at

<http://www.uos.harvard.edu/ehs/longwood/HarvardLongwoodLabWasteGuide.pdf>

- Dispose of as hazardous chemical waste.

EMEGENCY PROCEDURES

Refer to the emergency flip chart titled "*EHS Procedures and Response Guidelines*," posted in each laboratory and found on EHS's webpage at <http://www.uos.harvard.edu/ehs/longwood/>.

Chemical Spill:

- For small spills, follow chemical spill response guidelines above. Don protective clothing, extinguish all ignition sources, and carefully apply vermiculite or other appropriate spill absorbent material to the spill. Place in a durable and sealable container for disposal.
- For spill clean-ups, use Barricade™ or Responder™ gloves (*chemical breakthrough time > 8 hrs*). Viton™ or Silver Shield™/4H™ gloves are acceptable, as well. Do not wear nitrile gloves, due to the risk of direct or prolonged contact when cleaning up a spill.
- Respiratory protection may be necessary in the event of a large spill or release in a confined area.
- For a large spill, vacate the lab, deny further entry, and call EHS for assistance.

Fire:

- In the event of fire, evacuate and bar further entry. Activate fire alarm and leave the building.