Safety Precautions When Using RNA Extraction Kits

Many labs use RNA extraction kits to isolate total RNA from a wide variety of samples. Phenol-chloroform extractions, often using the trade-name reagent TRIzol®, are a common component of such kits. TRIzol® Reagent is a monophasic solution of phenol, guanidine isothiocyanate, and other proprietary components which facilitate the isolation of RNA.

While the kits are low hazard on their own, it's important to remember that mixing incompatible chemicals can produce high hazard by-product. There were incidents where researchers mixed bleach or strong acids with RNA extraction kit waste and produced hazardous gases.

We would like to bring your attention to the following safety steps when working with RNA extractions:

- ✓ As biological materials are already inactivated by TRIzol® and other cell-lysis related reagents (i.e. DNAzol, RNAzol or Tri-reagent, etc.), there is NO need to decontaminate them with bleach. Dispose this kind of chemical-containing biological waste as chemical waste directly.
- ✓ The additional step using bleach is a dangerous, unnecessary practice. TRIzol® and related chemical reagents contain guanidine hydrochloride or other acidic solutions such as those found in Qiagen kits are not compatible with bleach.
- ✓ Label all chemicals and solutions. A full chemical name and concentration is required for all hazardous materials.
- ✓ When working with TRIzol® and/or similar chemical reagents, always work in a chemical fume hood and wear lab coat, disposable gloves and eye protection. Change gloves frequently.
- ✓ Use disposable, sterile plastic ware and RNase-free pipette tips and tubes.
- ✓ Always use proper microbiological aseptic techniques when working with RNA.
- ✓ Use clear polypropylene disposable tubes when working with <2 ml volumes of TRIzol® Reagent. For larger volumes, use glass (Corex) or polypropylene tubes, and ensure that the tubes can withstand centrifugation at 12,000 × g with TRIzol® Reagent and chloroform. Do not use tubes that leak or crack.
- ✓ Use a reagent like RNase AWAY® to remove RNase contamination from work surfaces and non– disposable items such as centrifuges.
- ✓ Always read the SDS. There is a section for incompatible chemicals in SDS sheets.
- ✓ All solutions containing any amount of the chemicals listed below must be collected as hazardous waste.

DO NOT MIX BLEACH WITH TRIZOL® OR OTHER INCOMPATIBLE CHEMICALS.
If any injury occurs, immediately contact Public Safety (954-262-8889) and EHS.

EHS has developed the following chemical **Safety Fact Sheet** for some of the commonly used hazardous materials used in phenol-chloroform extractions.

1. **TRIzol®**
   - TRIzol® Reagent contains phenol (toxic and corrosive) and guanidine isothiocyanate (an irritant) and may be a health hazard if not handled properly. Avoid direct contact with TRIzol® Reagent, as direct contact of skin, eyes, or respiratory tract with TRIzol® Reagent may cause chemical burns to the exposed area.
   - Both Lysis Buffer and Wash Buffer I contain guanidine isothiocyanate (an irritant). This chemical is harmful when in contact with the skin, or when it is inhaled or ingested.
   - Solutions containing ethanol are considered flammable. Use appropriate precautions when using this chemical.

2. **Phenol**
   - Phenol, one of the two components of TRIzol®, is corrosive to the skin and eyes and is toxic by inhalation, ingestion, and dermal exposure.
   - Contact with phenol will reduce the efficacy of nitrile gloves. Be alert for any exposure of your gloves to phenol, and if you observe an exposure, change gloves immediately, dispose of the contaminated gloves as hazardous waste, and wash your hands thoroughly with soap and water.
   - When working with large quantities of phenol, butyl-rubber gloves should be worn instead of nitrile.

3. **Guanidinium thiocyanate**
   - Guanidinium thiocyanate, one of the two components of TRIzol®, is corrosive to the skin and eyes and is toxic by inhalation, ingestion, and dermal exposure.
   - If guanidinium thiocyanate is mixed with bleach, several toxic gases will be produced.

4. **Chloroform or bromochloropropane**
   - *Bromochloropropane is a preferable alternative to chloroform because it is less toxic and less volatile. Moreover, bromochloropropane gives better phase separation, which results in improved removal of genomic DNA from total RNA preparations and, therefore, better purification of RNA.*
   - Chloroform is carcinogenic, teratogenic, and toxic by inhalation and ingestion.
   - Contact with chloroform will reduce the efficacy of nitrile gloves. Be alert for any exposure of your gloves to chloroform, and if you observe an exposure, change gloves immediately, dispose of the contaminated gloves as hazardous waste, and wash your hands thoroughly with soap and water.
   - When working with large quantities of chloroform, fluorinated rubber gloves should be used instead of nitrile.

5. **Isoamyl, isopropyl, and ethyl alcohols**
   - All three alcohols commonly used in RNA extraction are flammable. Isoamyl alcohol causes eye damage. 70% ethanol may have other hazards associated with it depending on possible additives (e.g., methanol).
   - All work with isoamyl alcohol should be performed in a certified chemical fume hood. Work with large quantities (>500 mL) of isopropyl and/or ethyl alcohols should be performed in a certified chemical fume hood. Note that biosafety cabinets will NOT provide adequate respiratory protection and may increase the risk of fire for work with flammable materials.

6. **Diethyl pyro carbonate (aka DEPC)**
   - DEPC is a combustible material and will react with a wide array of chemicals including strong acids, strong bases, ammonia, strong oxidizing agents, and strong reducing agents.
   - Avoid mixing DEPC with any chemicals not required for your procedure.
   - Contact with DEPC will reduce the efficacy of nitrile gloves. Be alert for any exposure of your gloves to 1-bromo-3-chloropropane, and if you observe an exposure, change gloves immediately, dispose of the contaminated gloves as hazardous waste, and wash your hands thoroughly with soap and water.
   - When working with large quantities of DEPC, butyl rubber gloves are recommended.

7. **1-bromo-3-chloropropane**
   - While not as commonly used as the materials above, some RNA extraction procedures recommend the use of this material.
   - 1-bromo-3-chloropropane is toxic by inhalation and ingestion.
   - When working with large quantities of 1-bromo-3-chloropropane, butyl rubber gloves are recommended. Fluorinated rubber gloves are another suitable option.
   - Contact with 1-bromo-3-chloropropane will reduce the efficacy of nitrile gloves. Be alert for any exposure of your gloves to 1-bromo-3-chloropropane, and if you observe an exposure, change gloves immediately, dispose of the contaminated gloves as hazardous waste, and wash your hands thoroughly with soap and water.

8. **2-mercaptoethanol (aka beta-mercaptoethanol aka BME)**
   - While not as commonly used as the materials above, some RNA extraction procedures recommend the use of this material.
   - 2-mercaptoethanol is flammable, corrosive to the skin and eye, a skin sensitizer (i.e., can cause allergies), and toxic by inhalation, ingestion, and particularly dermal exposure.
   - When working with 2-mercaptoethanol at any quantity above 1 mL, thick (0.6 mm) latex gloves or butyl rubber gloves should be worn.
   - Contact with 2-mercaptoethanol will reduce the efficacy of nitrile gloves. Be alert for any exposure of your gloves to 2-mercaptoethanol, and if you observe an

exposure, change gloves immediately, dispose of the contaminated gloves as hazardous waste, and wash your hands thoroughly with soap and water.

If you have any questions about the safe handling and storage of these or other chemicals, please contact EHS at EHS@nova.edu and ask to speak to a lab safety representative.

REFERENCES

1. Cold Spring Harbor Protocols: Cold Spring Harbor Protocols
2. TRIzol® Reagent: TRIzol® Reagent User Guide - Pub. no. MAN0001271 - Rev. A.0
3. Boston University – EHS
4. Stanford University – RNA extraction Safety
5. Yale University – TRIzol® RNA Isolation Protocol
6. Thermo Fisher Scientific Protocols: TRIzol®® Plus RNA Purification Kit | Thermo Fisher Scientific - US