



Guidelines
For the
Safe Use & Disposal
Of Ethidium Bromide

Department of Environmental Health & Safety

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Ethidium bromide (EtBr) is a potent mutagen that has been used for many years for the visualization of nucleic acids in agarose gels. This material fluoresces a red-orange color under ultraviolet light and with increased fluorescence when bound to double-stranded DNA. EtBr is typically purchased in powder or solution form and is soluble in water. The crystal or powder form is odorless and appears dark red in color.

The powder form is considered an irritant to the upper respiratory tract, eyes and skin. EtBr is strongly mutagenic, causing living cell mutations. Even though there is no evidence at this time of human carcinogenicity or teratogenicity, this material should be considered a possible carcinogen or teratogen and all possible precautions taken to minimize potential exposures. While it is not specifically listed as a hazardous waste, its mutagenic properties may present a hazard if not managed properly.

When purchasing EtBr make sure that you purchase the smallest amount possible for your use. Stock quantities of unused EtBr should be returned to the supplier if possible. You should also check with other investigators in your area to see if they could use the product. When all other means of distribution have been eliminated then you should call Environmental Health & Safety (EHS) to pick-up the unused product for disposal.

SAFE USE

I. Personal Protection Equipment (PPE)

As in any laboratory, never eat, drink, smoke, handle contact lenses or apply cosmetics where EtBr or other chemicals are present. An operational emergency eyewash and shower should be immediately accessible. Wear a lab coat with sleeves rolled down, chemical splash goggles (NOT safety glasses), and nitrile disposable gloves when working with concentrated EtBr. Even when working with diluted concentrations, nitrile gloves should always be worn. Never re-use disposable gloves. Leave lab coats, gloves, and other Personal Protective Equipment (PPE) in the lab when your work is complete. To prevent the spread of this or other chemicals outside of the lab, EtBr users should also wash their hands thoroughly with soap and water after removing their gloves, even if they are certain the gloves weren't punctured.

Confine all work with dry EtBr to a properly functioning, uncluttered chemical fume hood - be sure the hood sash is at the correct height (≤ 12 inches). Also, consider using pre-mixed solutions or pre-measured quantities. As with all hazardous substances, work on a disposable mat in the hood, and dispose of used mat properly after work is done. Whenever weighing dry EtBr, use a chamber or enclosed weighing station to reduce/eliminate cross drafts which can affect the performance of the scales and also eliminate the potential for airborne contamination.

If an ultraviolet (UV) light source is used in your work with EtBr, added caution is required. As a general rule, avoid exposing unprotected skin and eyes to intense UV sources. If the UV light is aimed upwards, wear goggles or glasses rated for UV short wave lengths when you are standing near the source. For prolonged work close to UV light boxes or other intense sources, it may be useful to wrap the end of the lab coat sleeves loosely with masking tape to prevent gaps where the wrist could be exposed. For low-intensity UV sources, the requirement for UV protection can be waived if the exposure to personnel has been measured and shown to be within permissible exposure levels. Contact the Radiation Safety Office (RSO) in EHS at 410-296-7593 if you need measurements of the UV levels in your lab.

II. Work Practices

When working with EtBr, try to minimize the potential for spills. Where practical, purchase ready-made stock solutions from chemical manufacturers in lieu of mixing your own solutions. If you prefer to mix your own solutions of EtBr, protect yourself by doing this process in an operable fume hood with the sash at the correct height. Perform all processes that may generate EtBr dusts or mists inside the fume hood to minimize inhalation exposures. Prevent accidents by keeping container sizes and quantities in the work area as small as possible and transporting small quantities of EtBr in a secondary container instead of carrying large quantities. Keep EtBr containers tightly closed when not actively pouring to or from the container. Whenever you transport gels, always use secondary containment (i.e., basins or trays) to contain spills and prevent building contamination.

All non-waste EtBr containers must be labeled in accordance with Federal and State Right-to-Know regulations. At a minimum, ***all*** EtBr containers must contain the following information:

- Name of the material
- Concentration
- Warnings
- Date solution was prepared, if appropriate
- Preparer's initials, if appropriate.

Employees and students working with EtBr should be familiar with these procedures and trained on its proper use, disposal and hazards by Principle Investigators ***prior*** to use.

III. Spill Procedures

In case of a small (≤ 1 gallon), free standing spill, absorb free standing liquid using paper towels and dispose of the contaminated towels as contaminated solid waste. Don't forget to put on appropriate PPE before cleaning up the spill. Use a UV light to locate any additional contamination. Using the decontamination solution and procedures in the Contaminated Equipment section below, decontaminate the spill area until no UV fluorescence remains and collect and separate contaminated clean-up materials (solids, liquids, etc.) for disposal as regulated hazardous waste through EHS.

In case of a large spill (> 1 gallon), immediately notify all lab personnel and evacuate the lab immediately. Post warning signs on all lab doors notifying others of the spill. If possible, try to contain the spill to prevent it from flowing down drains, under cabinets, etc. Do not track EtBr out of the immediate spill area. Contact EHS and prevent unnecessary entry into the room until EHS personnel arrive. Stand by to assist EHS in the clean-up.

IV. Emergency Procedures

If EtBr contacts the eyes, immediately flush them with copious amounts of cold water for at least 15 minutes. (If it is available, an emergency eyewash is the best and safest way to do this.) For skin contact, remove contaminated clothing and immediately wash the affected area with soap and copious amounts of cold or cool water. If a person inhales EtBr dust, move them to fresh air. **After any eye or internal exposure (inhalation or ingestion) to EtBr, the affected person should immediately seek medical evaluation.**

Waste Disposal

Unwanted solid EtBr, gels, and all working solutions must be disposed of through EHS. Disposal of EtBr via sink drains is strictly prohibited. Working solutions can be disposed of through EHS or may be rendered non-toxic as the last step of your experimental process.

Separate EtBr liquid waste from solid waste and place into separate waste containers and ensure that:

- Each waste container has a hazardous waste label (available from EHS) which is dated with the date waste is first placed into the container and legibly lists all waste ingredients (including water) and approximate concentrations. (Concentrations must total 100 %.)
- If potentially corrosive ($\text{pH} \leq 2.0$ or ≥ 12.5), list pH on hazardous waste label.
- All hazardous waste containers are tightly closed except when actively adding waste to the container.
- All EtBr waste containers are stored in appropriately sized secondary containment devices (SCD's). (Appropriately sized is defined as capable of containing 100% of the total waste volume contained by the SCD OR 100% of the capacity of the largest container stored within [whichever is greater].)

I. Electrophoresis Gels

Prior to the disposal of gels, separate the high concentration gels (>0.5 micrograms/milliliter [$\mu\text{g}/\text{ml}$] EtBr) from the low concentration gels (≤ 0.5 $\mu\text{g}/\text{ml}$):

- Place the high concentration gels into an appropriately labeled (i.e., "Waste Ethidium Bromide Gels - >0.5 $\mu\text{g}/\text{ml}$ ") hazardous waste container for disposal by EHS when full. Don't forget to date the hazardous waste label with the date waste is first put into the container. Wherever possible, high concentration gels should be allowed to thoroughly dry by evaporation prior to disposal in order to reduce disposal cost (charge is by weight).
- It is not necessary to dry the low concentration gels (≤ 0.5 $\mu\text{g}/\text{ml}$ EtBr) and they may be directly disposed of via traditional domestic waste disposal channels if double bagged and tightly tied in black or opaque trash bags. Do not use red or Special Medical Waste (SMW) bags for disposal of gels contaminated with ≤ 0.5 $\mu\text{g}/\text{ml}$ of EtBr.

II. Aqueous Solutions

Stock, rinse and wash solutions can be decontaminated or deactivated using the following methods or use the procedures published in the National Research Council's report, ***Prudent Practices in the Laboratory: Handling and Disposal of Chemicals***, or in other peer-reviewed scientific journals. EHS strongly recommends the charcoal filtration method of disposal.

Please Note: These procedures ONLY apply to aqueous EtBr solutions. EtBr solutions containing other regulated hazardous wastes can not be treated and MUST be disposed of through EHS.

1. Charcoal Filtration:

Filtering aqueous EtBr waste solutions through a bed of activated charcoal is a relatively simple and effective way to remove EtBr. The filtrate is non-hazardous and may be poured down the drain. There are two commercially available kits available for the filtration of aqueous EtBr wastes.

A. S&S Funnel Kit: This kit is available through VWR (VWR#28165-502) and uses a funnel and packaged charcoal disk to filter up to 10 liters of aqueous EtBr waste. Once the filter has expired, it should be packaged in a sealed bag (i.e., Zip-Lock) and disposed of through EHS.

To extend filter life, keep funnel covered when not in use.



B) Bio 101 “EtBr Greenbag Disposal Kit: This kit is available through BIO 101” (#2350- 200) and uses a charcoal “tea bag” to remove EtBr from aqueous waste solutions. The used “tea bag” is packaged into a sealed bag (i.e., Zip-Lock) and disposed of through EHS. One kit has the capacity to remove 500mg of EtBr from solutions (10mg EtBr/bag).



2. Chemical Deactivation

Chemical deactivation is not the preferred disposal method. However, if you choose to detoxify EtBr in your laboratory, hazardous waste regulations require that you perform the procedure at or near the point of EtBr use and waste generation (i.e., same room). Solutions containing EtBr can be deactivated and rendered non-hazardous by using the following method. Deactivation may be confirmed by using UV light to detect fluorescence.

A. Lunn & Sansone Method: For each 100 ml of aqueous EtBr solution:

- Add 5% hypophosphorous acid.
- Add 12 ml of 0.5 M sodium nitrite.
- Stir briefly and let stand for 20 hours.
- Adjust pH to 7-9 using sodium hydroxide.
- Pour down drain with copious amounts of water.

III. Crystals & Powders

Dispose of old or unwanted containers of EtBr crystals or powders as regulated hazardous waste through EHS.

IV. Contaminated Solids

Contaminated solids consist of gloves, paper towels, bench paper and other non-hazardous labware items and should be collected for disposal as a regulated hazardous waste through EHS. Solid waste containers must not contain any liquids. EHS has appropriate solid waste disposal containers.

V. Contaminated Labware

Potentially dangerous needles, scalpels, pipettes and other sharps contaminated with EtBr. Contaminated sharps must not contain any liquids and should be disposed of directly into an approved sharps container.

Disposable glassware incidentally contaminated with EtBr should be disposed of in a puncture resistant broken glass container and disposed of as non-regulated domestic waste. (Incidentally contaminated is defined as no visible contamination but has been in contact with EtBr solids or solutions.) Grossly contaminated (visibly contaminated) glassware should be washed with bleach before disposal in a broken glass container.

Centrifuge and test tubes contaminated with EtBr should first be emptied and the liquid disposed of in accordance with the procedures above. Empty incidental tubes can be disposed of as non-hazardous domestic waste. Grossly contaminated (visibly contaminated) tubes should be collected for disposal with other contaminated solids as a regulated hazardous waste through EHS.

VI. Contaminated Equipment

Lab equipment and lab surfaces contaminated with solid EtBr or aqueous solutions of $\geq 0.5\mu\text{g/ml}$ EtBr should be thoroughly decontaminated prior to re-use using the following methods:

- Use a UV light to locate the contamination.
- Prepare a decontamination solution by mixing 4.2 grams of sodium nitrite and 20 ml of 5% hypophosphorous acid in 300ml of water.
- Wash the contaminated area with a paper towel soaked in the decontamination solution. Repeat the wash with fresh wet paper towels 5 additional times. After cleaning up, place all the used paper towels into the unused decontamination solution and allow them to soak for 1 hour.

- Check the completeness of the decontamination using a UV light. Repeat the decontamination procedures with a fresh decontamination solution if any fluorescence remains.
- Collect and separate contaminated cleanup materials (solids, liquids, etc) for disposal as regulated hazardous waste through EHS.

V. *Waste Minimization*

State and Federal hazardous waste regulations require that hazardous waste generators minimize the generation of hazardous wastes. As an EtBr generator, it is your responsibility to minimize wherever possible, the generation of EtBr wastes. The following are suggestions for minimizing EtBr wastes:

- Wherever possible, substitute less or non-toxic stains for Ethidium Bromide in the identification of DNA
- Recently, new fluorescent dyes have been developed that manufacturers, such as Molecular Probes Inc. and FMC Bioproducts, claim are less toxic and can detect nucleic acid components at lower concentrations than EtBr. However, they still must be handled and disposed of in the same manner as EtBr.
- Use commercially available EtBr extractors/filters to remove EtBr from buffers and other aqueous solutions.
- Use dilute rather than concentrated EtBr solutions wherever possible.
- Use micro or semi-micro scale techniques.