



GUIDE FOR ETHIDIUM BROMIDE USE AND DISPOSAL

NOTE: Ethidium bromide is a chemical and should NOT be treated or labeled as a biohazard.

Ethidium Bromide (EtBr; *3,8 diamino-5-ethyl-6-phenyl phenanthridinium bromide, dromilac*, CAS #1239-45-8), commonly used in research laboratories as a stain for the visualization of nucleic acids in electrophoresis gels, is a toxic chemical and a potent mutagen. When used in nucleic acid staining, ethidium bromide fluoresces a red-orange to pink color under ultraviolet light and with increased fluorescence when bound to double-stranded DNA. While it is not specifically regulated as a hazardous waste by the EPA, the mutagenic properties may present health hazards and disposal concerns if it is not managed properly in the laboratory.

I. Required PPE:

Always wear a lab coat, gloves, and appropriate protective eyewear when handling ethidium bromide and/or ethidium bromide containing material. Ensure the lab coat is buttoned or zipped closed. Proper skin and eye protection are also needed when an ultraviolet (UV) light source is used while working with ethidium bromide. Avoid exposing unprotected skin and eyes to intense UV sources. A face shield is suggested if the UV source is pointing upwards.

II. Guidelines for Ethidium Bromide Disposal:

- a. Stock Solutions: Stock solutions of ethidium bromide typically contain higher concentrations of ethidium bromide (approximately 10 mg/ml). Follow the [TTU Hazardous Waste Management and Satellite Accumulation Area Guide](#) for proper disposal of all unwanted stock solutions of ethidium bromide.
- b. Electrophoresis Gels and Buffers: Solid ethidium bromide waste (e.g., gels) typically contains 3 –5 ug/ml of ethidium bromide. Liquid ethidium bromide waste (e.g., buffers) typically contains less than 0.5 ug/ml of ethidium bromide.
 - i. Gels of ethidium bromide needs to be dried out. Ethidium bromide gels, contaminated gloves, and other equipment as chemical hazardous waste. These materials would be considered a dry solid waste. These materials need to be placed into a leak-proof seal container.
 - ii. Buffers with less than 0.5 ug/ml of ethidium bromide can be poured down the sink after it is treated via charcoal filtration prior to drain disposal is required (See methods listed below).
 - iii. Buffers with unknown concentrations or concentration of >0.5 ug/ml of ethidium bromide must be disposed of as hazardous chemical waste.
 - iv. Any ethidium bromide solution containing heavy metals, solvents, cyanides, sulfides, acids, or bases must be disposed of as hazardous chemical waste

- v. Follow the [TTU Hazardous Waste Management and Satellite Accumulation Area Guide](#) for proper disposal of hazardous chemical waste.
- c. Sharps (e.g., needles, syringes, razor blades) contaminated with ethidium bromide must be discarded in an appropriate puncture proof plastic container. The container cannot be red and cannot have the biohazard symbol on it.
 - i. Follow the [TTU Hazardous Waste Management and Satellite Accumulation Area Guide](#) for proper disposal of hazardous chemical waste.

III. Charcoal Filtration of Aqueous Ethidium Bromide Waste:

a. Charcoal Filtration:

Dilute aqueous solutions containing <0.5 mg/ml ethidium bromide can be filtered or deactivated using one of the methods described below. EHS strongly recommends charcoal filtration over chemical deactivation. If you do not want to filter or deactivate the solutions, you must manage these solutions as a chemical hazardous waste. Filtering the aqueous ethidium bromide waste solution, free of other contaminants, through a bed of activated charcoal is a relatively simple and effective method for removal of ethidium bromide. The filtrate may be poured down the sink drain.

b. The Green Bag:

One simple charcoal filtration method is the Green Bag, manufactured by BIO 101. The Green Bag® Kit allows rapid and trouble-free concentration of ethidium bromide from large volumes of solutions into a small "tea" bag containing activated carbon which is then conveniently disposed of along with other solid hazardous chemical wastes. One kit has the capacity to remove 500 mg of ethidium bromide from solutions (10 mg EtBr/bag).

- Place the Green Bag into the ethidium bromide solution.
- Allow to stand for the allotted time.
- Pour filtrate down the drain.
- Place charcoal filter in a sealed bag (e.g., zip-lock) for chemical hazardous waste disposal.

Ethidium Bromide Green Bag Disposal Kit (cat. # 112350200) is available from www.mpbio.com.

c. Powdered Activated Charcoal:

Powdered activated charcoal can be used for buffers containing less than or equal to 0.5 ug/ml of ethidium bromide. Sigma-Aldrich (Cat# C3014) is available from www.sigmaldrich.com.

- Add 100 mg of powdered activated charcoal for each 100 ml of waste buffer solution.
- Store the solution for one hour at room temperature, shaking it intermittently. Filter the solution through a Whatman No. 1 filter and drain dispose of the filtrate.

- Seal the filter and activated charcoal in a plastic bag (e.g., zip-lock) for chemical hazardous waste disposal.
- d. Funnel Kit:
- Fisher Scientific sells a commercial filter funnel kit that uses a packaged charcoal disk that is graduated for easily tracking the amount of aqueous solution calculated for a fixed quantity of ethidium bromide residue. This is particularly useful for labs that generate large amounts of ethidium bromide solution at a time. The following kit is available through Fisher Scientific (# 10448031) www.fishersci.com.
- Filter the ethidium bromide solution through the charcoal filter.
 - Pour filtrate down the drain.
 - Place charcoal filter in a sealed bag (e.g., zip-lock) for chemical hazardous waste disposal.

IV. Contaminated Equipment

- a. Lab equipment and lab surfaces contaminated with solid ethidium bromide or aqueous solutions of $>0.5\mu\text{g/ml}$ ethidium bromide should be thoroughly decontaminated prior to re-use using the following methods:
- i. Use a UV light to locate the contamination.
 - ii. Prepare a decontamination solution by mixing 4.2 grams of sodium nitrite and 20 ml of 5% hypophosphorous acid in 300ml of water.
 - iii. 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.
 - iv. Check the completeness of the decontamination using a UV light. Repeat the decontamination procedures with a fresh decontamination solution if any fluorescence remains.
 - v. Collect and separate contaminated cleanup materials (solids, liquids, etc) for chemical hazardous waste disposal.

V. Waste Minimization

- a. State and Federal hazardous waste regulations require that hazardous waste generators minimize the generation of hazardous wastes. As an ethidium bromide generator, it is your responsibility to minimize wherever possible, the generation of ethidium bromide wastes. The following are suggestions for minimizing ethidium bromide wastes:
- i. Wherever possible, substitute less or non-toxic stains for ethidium bromide in the identification of DNA.
 - ii. Fluorescent dyes have been developed that claim they are less toxic and can detect nucleic acid components at lower concentrations than ethidium bromide. However, they still must be handled and disposed of in the same manner as ethidium bromide.
 - iii. Use commercially available ethidium bromide extractors/filters to remove ethidium bromide from buffers and other aqueous solutions.
 - iv. Use dilute rather than concentrated ethidium bromide solutions wherever possible.
 - v. Use micro or semi-micro scale techniques.