

Guidelines for Ethidium Bromide Waste Management & Disposal

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1. Background

Ethidium bromide (*3,8 diamino-5-ethyl-6-phenyl phenanthridinium bromide, dromilac, CAS #1239-45-8*), is a compound used in many UTK laboratories. Ethidium bromide is available as a dark red, crystalline, non-volatile solid and is moderately soluble in water. Since it fluoresces readily with a reddish-brown color when exposed to ultraviolet (UV) light and with increased brightness when bound to double stranded-DNA and single-stranded RNA, it is commonly used in gel electrophoresis applications for visualization of these molecules. For these applications, liquid ethidium bromide solutions are incorporated into the electrophoresis gel as a dye for the DNA, RNA, or other molecules to be visualized.

2. Safety Considerations

Ethidium bromide is mutagenic and moderately toxic and must be handled with care. The powder form is considered an irritant to the upper respiratory tract, eyes, and skin. Ethidium bromide is thought to act as a mutagen because it bonds in double stranded DNA, thereby deforming the molecule. This is believed to block or trip biological processes occurring on DNA. Preparation of stock solutions and any operations capable of generating ethidium bromide dust or aerosols should be conducted in a fume hood to prevent inhalation. Nitrile gloves, a lab coat, and eye protection should be worn at all times, as with working with any hazardous material.

When working with ethidium bromide, minimize the potential for spills. Where practical, purchase pre-mixed stock solutions from chemical manufacturers in lieu of preparing solutions. If solutions of ethidium bromide must be prepared, consider performing this process in a fume hood. Perform all processes that generate ethidium bromide dusts or mists inside the fume hood to minimize inhalation exposures. Prevent accidents by transporting small quantities of ethidium bromide in secondary containment.

When an ultraviolet light source is used in work with ethidium bromide, added caution is required. As a general rule, avoid exposing unprotected skin and eyes to intense UV sources.

3. Ethidium Bromide Waste Disposal

Ethidium bromide may present a hazard if it is poured down the drain untreated or placed in the trash. Ethidium bromide is not regulated by the EPA as hazardous waste, but UTK manages ethidium bromide waste as a special waste, and should be managed the same as a hazardous waste. Any ethidium bromide waste should not be poured down the drain, or thrown in the trash, unless the waste has been deactivated or filtered. The following are the recommended disposal procedures for ethidium bromide:

A. Electrophoresis Gels, Contaminated Gloves and Other Equipment

Manage ethidium bromide gels, contaminated gloves and other equipment (DRY wastes) just as you would a chemical hazardous waste. There is one exception: you can dry the gels under the hood in an open container before bringing to the waste room for disposal. (NOTE: The gels must not have running liquid in the bottom of the bag), but this does not apply to other chemical waste. EHS prefers the use of sealable, disposable plastic containers to store ethidium bromide gel waste, or wide mouth jars. Please note that these containers will not be given back to you (they are for one time use), nor does EHS supply these containers. Sturdy bags can also be used as well. In either case, there should be no free flowing liquids in these containers when they are brought to the waste room for subsequent disposal.

Figure 1: Example of disposable plastic container



When it is time for disposal, place the container or bag in a secondary container such as a bucket, cardboard box, or secondary sturdy bag and place a UT hazardous waste label on the outside of the package, with the contents identified as ethidium bromide on the label. Manage ethidium bromide waste containers like any other hazardous waste - it must be stored a container that is labeled and closed unless you are actively adding waste to the container, or drying the gels. Once the container is filled, you can bring the waste to one of the waste rooms for disposal.

NOTE - Ethidium bromide waste that is not mixed with a biological hazard DOES NOT go in red bags or red containers, should not be labeled with a biosafety symbol, and especially should not be treated in an autoclave. For mixed ethidium bromide and biohazard waste contact EHS at 4-5084, or the UT Biosafety Officer at 4-1938 for guidance.

B. Sharps Contaminated with Ethidium Bromide

Contaminated needles, syringes, etc. must be discarded into a puncture-proof plastic container (must not be red and must not have a biohazard symbol on it) with a lid that closes. It must be labeled as ethidium bromide waste, with a UT hazardous waste label. Do not mix these chemically contaminated sharps in a red biohazard sharps container that may go in the autoclave. Autoclaving chemical waste creates exposure to the chemicals as they heat and volatilize. Dispose of the container as chemical hazardous waste and bring to the waste room for disposal.

C. Ethidium Bromide Solutions

- 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 hazardous waste (UT hazardous waste label) and bring to the waste room.
- Concentrated solutions (>0.5 mg/ml) should be collected for disposal as hazardous waste.
- Any solutions that also contain heavy metals, organics, cyanides or sulfides should be collected and disposed of as hazardous waste.

1. Charcoal Filtration

Filtering the aqueous ethidium bromide waste solutions, 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 then be poured down the drain.

There are two simple kits available for charcoal filtration:

a. Funnel Kit

Schleicher and Schuell supply a commercial filter funnel kit that uses a packaged charcoal disk that is graduated for easily tracking the amount of aqueous solution calculated for fixed quantities of ethidium bromide residue. This product is recommended for dilute solutions such as electrophoresis gel staining buffer (0.5X TBE/0.5 ug/ml ethidium bromide) and is particularly useful for labs that generate large amounts of solutions at a time. The kit is available through Fisher Scientific, Schleicher and Schuell, or VWR.



- 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) and place with ethidium bromide gels for hazardous waste disposal.

b. The Green Bag

Another 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 along with other solid hazardous wastes. One kit has the capacity to remove 500 mg of ethidium bromide from solutions (10mg Ethidium bromide per "tea bag").



- Place the Green Bag into the ethidium bromide solution.
- Allow to sit for the allotted time.
- Pour filtrate down the drain.
- Dispose of the Green bag with Ethidium bromide gels for hazardous waste disposal.

4. Ethidium Bromide Alternatives

Consider switching to less-toxic alternatives to ethidium bromide (SYBR Safe™ DNA gel stain, for example) to reduce potential hazardous exposures in the lab. Disposal of most ethidium bromide alternatives must be managed by EHS, in a manner similar to that described above for ethidium bromide. Contact EHS for specific guidance if you have questions regarding proper disposal of ethidium bromide alternatives.

5. Ethidium Bromide Spill Cleanup

For small spills (<20 ml) of concentrations up to 10 mg/ml:

- Absorb with paper towel and then treat area with 70-95% ethanol/isopropanol and wipe up—all to be collected as hazardous waste.
- Repeat as necessary (if still fluorescing).
- Final rinse with soap and water (paper towels for this step were discarded into regular trash).
- DO NOT clean ethidium bromide spills with bleach solutions.

If you don't feel comfortable cleaning up the spill, please call EHS at 974-5084 during normal office hours and UT Police at 974-3111 after hours.