

Ethidium Bromide Use and Disposal

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

Ethidium Bromide (EtBr), 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, the mutagenic properties may present health hazards and disposal concerns if it is not managed properly in the laboratory.

Required PPE:

Always wear a lab coat, gloves, and appropriate protective eyewear when handling ethidium bromide and/or ethidium bromide containing material. 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.

Guidelines for Ethidium Bromide Disposal:

Stock Solutions: Stock solutions of ethidium bromide typically contain higher concentrations of ethidium bromide (approximately 10 mg/ml). Contact the Office of Environmental Safety & Services for collection and proper disposal of all unwanted stock solutions of ethidium bromide.

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.

- Gels with less than 0.5 ug/ml of ethidium bromide can be bagged and placed in the regular laboratory trash
- Buffers with less than 0.5 ug/ml of ethidium bromide can be released to the sewer; however, charcoal filtration prior to drain disposal is **STRONGLY** recommended (See methods listed below)
- Gels and buffers greater than or equal to 0.5 ug/ml of ethidium bromide must be stored in closed, labeled containers and collected by the Office of Environmental Safety & Services for appropriate chemical waste disposal or the buffer solution can be filtered properly prior to drain disposal (See methods listed below)
- Gels or buffers with unknown concentrations of ethidium bromide must be collected by the Office of Environmental Safety & Services for proper disposal or one of the methods listed below must be used to remove/deactivate/neutralize the ethidium bromide prior to drain disposal.
- Any ethidium bromide solution containing heavy metals, solvents, cyanides, sulfides, acids, or bases must be disposed of as hazardous chemical waste

- Sharps (e.g., needles, syringes, razor blades) contaminated with ethidium bromide must continue to be discarded in an appropriate sharps container
- Grossly contaminated materials (e.g., gloves, paper towels, etc) must be collected in closed, labeled containers (e.g., sealable plastic bag) and collected by the Office of Environmental Safety & Services for appropriate chemical waste disposal

Methods for the Removal/Deactivation/Neutralization of Ethidium Bromide:

Ethidium bromide waste (e.g., gels, buffers) should be safely collected and clearly labeled at all times. The easiest and safest option for ethidium bromide waste disposal is collection by the Office of Environmental Safety & Services as hazardous chemical waste. However, as a waste generator at Saint Louis University you have the responsibility of minimizing the waste you create. The Office of Environmental Safety & Services strongly recommends one of the following treatment methods prior to pouring ethidium bromide liquid waste down the drain.

1.) Charcoal Filtration

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.

There are three simple options available for charcoal filtration:

□ **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 waste collection by the Office of Environmental Safety & Services.

Ethidium Bromide Green Bag Disposal Kit (cat. # 2350-200) is available from <http://www.qbiogene.com>

□ Powdered Activated Charcoal

Powdered activated charcoal can be used for buffers containing less than or equal to 0.5 ug/ml of ethidium bromide.

- 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 collection by the Office of Environmental Safety & Services

□ 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). <https://www1.fishersci.com/index.jsp>



- 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 waste pickup by the Office of Environmental Safety & Services.

2.) Chemical Neutralization

Solutions containing ethidium bromide can be deactivated, neutralized and poured down the drain. Deactivation may be confirmed using UV light to detect fluorescence. Most ethidium bromide users are familiar with using household bleach as a method of deactivation. However, in 1987, Lunn and Sansone studied eight methods for destroying ethidium bromide. By using mutagenic assay techniques they showed that as much as 5% of the activity of ethidium bromide (or a subsequent mutagen formed by the oxidation) survived treatment with sodium hypochlorite. Their studies showed the following protocol to be the best method for destroying mutagenic activity in ethidium bromide solutions (Lunn, G. and E. Sansone, *Analytical Biochemistry*, vol. 162, pp. 453-458, 1987).

□ Lunn and Sansone Method

For each 100 ml of ethidium bromide solution (0.5 mg/ml):

- Add 20 ml (5%) hypophosphorus acid solution.
- Add 12 ml of 0.5 M sodium nitrate solution.
- Stir the mixture briefly and let stand for 20 hours
- Adjust pH to 4-9 using sodium bicarbonate and pour down the drain.