

EH&S Guidelines for Ethidium Bromide Use and Disposal

Introduction:

Ethidium Bromide (EtBr) is commonly used in research laboratories as a stain for the visualization of nucleic acids in electrophoresis gels. When used for 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. The NIH Material Safety Data Sheet for EtBr states: "Warning: This compound is moderately toxic and strongly mutagenic." Although ethidium bromide is highly mutagenic, it does not meet the RCRA regulatory definition of hazardous waste (40 CFR 261). This does not mean that RCRA has no authority over the disposal of this compound. If managed improperly, ethidium bromide does meet the definition of hazardous waste according to 42 U.S.C. 6903 and would be subject to the imminent and substantial endangerment provisions of RCRA 7003, 42 U.S.C. 6973. 42 U.S.C. 6903 defines the term "hazardous waste" to mean a solid waste, or combination of solid wastes, which because of its quantity, concentration, or physical, chemical, or infectious characteristics may:

1. Cause, or significantly contribute to an increase in mortality or an increase in serious irreversible, or incapacitating reversible, illness; or
2. Pose a substantial present or potential hazard to human health or the environment when improperly treated, stored, transported, or disposed of, or otherwise managed.

NOTE: The EPA strongly suggests managing this waste as hazardous and employing a permitted hazardous waste transporter and treatment or disposal facility to ensure proper disposal, even though it does not meet the regulatory definition of hazardous waste. Other nucleic acid stains, such as SYBR™ dyes, propidium iodide, DAPI, Hoescht, etc., have a range of hazards affiliated with their use/disposal from cytotoxic to highly mutagenic. EH&S strongly recommends that all nucleic acid stains and their affiliated wastes (solid and liquid) be treated as hazardous and managed as outlined in this document.

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.

Preferred Method of Collection and Disposal:

1) *Electrophoresis Gels:*

All gels, filters, and other solid materials containing EtBr should be placed in a container, such as a cardboard box or sealable plastic tub, that is lined with a plastic bag (*DO NOT PLACE IN BIOHAZARDOUS WASTE BAGS*) and the EH&S yellow hazardous waste label must be attached and properly filled out. When full, the container must be disposed of through EH&S as a hazardous waste by visiting <http://ehs.wustl.edu/hazmats/hazwaste> and filling out the Chemical Removal Request Form.

2) *Aqueous Solutions:*

EtBr **IS NOT** allowed to be disposed of down the sink. All EtBr solutions regardless of concentration must be collected in an appropriate, sealable container for liquids and the EH&S yellow hazardous waste label must be attached and properly filled out. When full, the container must be disposed of through EH&S as a hazardous waste by visiting <http://ehs.wustl.edu/hazmats/hazwaste> and filling out the Chemical Removal Request Form.

Alternative Method for Collection and Disposal:

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. Prior to drain disposal, check for fluorescence by using a UV light or spectrophotometer to ensure complete removal of EtBr. When the filter is saturated, the charcoal must be disposed of through EH&S.

There are three simple options available for charcoal filtration:

1) 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), label with an appropriately filled out the yellow hazardous waste sticker, and dispose of through EH&S.

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

2) 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. Filters 10x 1L of liquid at 0.5mg/mL
- Pour filtrate down the drain.
- Place charcoal filter in a sealed bag (e.g., zip-lock), label with an appropriately filled out the yellow hazardous waste sticker, and dispose of through EH&S.

3) Powdered Activated Charcoal

Powdered activated charcoal can be used for buffers containing less than or equal to 0.5 µg/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), label with an appropriately filled out the yellow hazardous waste sticker, and dispose of through EH&S.

Can be purchased through Sigma <http://www.sigmaaldrich.com>

Summary of Disposal Procedures for Ethidium Bromide

Ethidium Bromide wastes are prudently managed by Washington University EH&S to minimize human and environmental exposure. Please follow the instructions listed in the following table when handling ethidium bromide and make sure that all wastes are appropriately labeled with the yellow hazardous waste sticker:

Waste Stream	Description	Waste Disposal Procedure
Buffer	Typically contain very small concentrations of EtBr (<0.5 mg/L)	Dispose as hazardous waste. Contact EH&S for a waste pick-up. Alternatively, filtration of the buffer through activated charcoal is acceptable.
Stock solutions	Typically contain higher concentrations of EtBr (1–10 mg/ml)	Dispose as hazardous waste in original container. Contact EH&S for a waste pick-up.
Gels	Typically contain lower concentrations of EtBr (3–5 mg/L)	Place gels in a container (cardboard box or sealable plastic tub) that is lined with a plastic bag. Dispose of as hazardous waste. Contact EH&S for a waste pick-up.
Contaminated Debris	Gloves, spill cleanup materials, and other lab supplies contaminated with EtBr	Broken glassware and sharps must be placed in puncture-resistant containers. Other debris may be placed in clear, labeled bags. Contact EH&S for a waste pick-up.
Crystals and powders	Typically pure or concentrated EtBr	Dispose of EtBr crystals and powders through EH&S. Contact EH&S for a waste pick-up.