

Ethidium Bromide

Ethidium bromide is a potent mutagen that is commonly used in many molecular biology laboratories. It is strongly mutagenic causing living cell mutations. It is also toxic and may be fatal if swallowed, inhaled or absorbed through the skin. Less exposures may cause coughing, sneezing, and irritation of the skin, eyes and respiratory tract. Even though there is no evidence at this time of human carcinogenicity or teratogenicity, this material should be handled as such. The toxic and mutagenic properties of this substance are of great safety concern and require special care in handling and waste disposal. Ethidium bromide is deemed a hazardous material and the waste is regulated by the *State of Michigan Act 451 Part 121*.

Work Practices

Ethidium bromide is typically purchased in powder form and is soluble in water. When practical, try to purchase ready-made stock solutions from chemical manufacturers in lieu of mixing your own solutions. If you prefer to mix your own solutions of ethidium bromide, protect yourself by working in a fume hood. All procedures that generate ethidium bromide dusts, mists, or aerosols must be performed inside a fume hood. Wear a lab coat, chemical splash goggles, and nitrile gloves when working with ethidium bromide. Also when working with ethidium bromide, try to minimize the potential for spills.

Waste disposal

Ethidium bromide waste streams typically include:

Buffer solutions

Gels

Stock solutions

Crystals, powders and

Contaminated debris

Electrophoresis gels

Typically the gels contain trace amounts of ethidium bromide and do not pose a hazard. Higher concentrations are detected when the color of the gel is dark pink or red and should *never* be placed in laboratory trash regardless of the estimated amount of ethidium bromide.

- Less than 0.1% ethidium bromide (pale pink in color): place in laboratory trash
- More than or equal to 0.1% ethidium bromide: gels can be collected and disposed through EH&S as hazardous chemical waste.
 - Simply log-on to the EH&S website under Chemical safety and complete the form for a pickup
- Gels also can be collected in a fume hood and allowed to dry by evaporation to reduce the volume of solid waste.
 - Collect in waste containers and or bags designated for this purpose. Double bag if bags are used.
 - Keep waste containers/bags in secondary container, closed when not in use, and labeled "Hazardous Waste, Ethidium Bromide."
 - Do not place gels in bucket without liners.
- All dark pink or red gels should be placed in a sealed container and dispose as hazardous waste regardless of the estimated amount.

Ethidium Bromide Solutions

- Aqueous solutions containing $<5\mu\text{g/ml}$ ethidium bromide can be released to the drain.
- Aqueous solutions containing $>5\mu\text{g/ml}$ ethidium bromide should be deactivated or filtered using one of the procedures below.
- All ethidium bromide solutions that are deactivated should be neutralized and poured down the drain with copious amounts of water.
- Deactivation may be confirmed using UV light to detect fluorescence. (Wear UV protection).
- The recognized methods for deactivation are as follows:

Amour Method

This is the simplest and most common method. Please be advised that one study found that traces of the mutagenic mixture remained when using this method. Therefore, it is highly recommended to confirm deactivation with a UV light to detect fluorescence.

(Lunn, G. and E. Sansone, *Analytical Biochemistry*, vol. 162, pp. 453-458, 1987).

This procedure **must** be performed in a fume hood.

- Combine equal amounts of ethidium bromide solution and *fresh* household bleach
- Stir constantly for four hours or let sit for 2-3 days.
- Adjust pH to 4-9 with sodium hydroxide.
- Test the final solution with a UV light to ascertain that the EB is destroyed. Remember to wear UV protection.
- Of the final solution, drain dispose 1 part solution with 20 parts tap water

Lunn and Sansone Method

This procedure should be performed in a fume hood.

For each 100ml of ethidium bromide solution:

- Add 20 ml 5% hypophosphorus acid solution
- Add 12 ml of 0.5 M sodium nitrate.
- Stir mixture and let stand for 20 hours.
- Adjust pH to 4-9 using sodium hydroxide.
- Pour down drain with copious amounts of water.

Quillardet and Hoffnung Method

This method uses 0.5 M potassium permanganate and 2.5M hydrochloric acid. Since chlorine gas may be released in significant concentration, EH&S does not recommend using this method.

Charcoal Filtration

Filtering the aqueous ethidium bromide waste solutions, through a bed of activated charcoal is a relatively simple effective method. Once the ethidium bromide has been removed, the filtrate may be poured down the drain. Commercial filter funnel kits are available that use a packaged charcoal disk that is graduated for easily tracking the amount of aqueous solution calculated for fixed quantities of ethidium bromide residue. Procedure includes:

- Filter the ethidium bromide solution through charcoal filter.
- Pour filtrate down the drain.
- Place the charcoal filter in a sealed bag (i.e. zip-lock) and place the bag in a biohazardous waste container.

There are two simple kits available for charcoal filtration:

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 a fixed quantities of ethidium bromide residue. This is particularly useful for labs that generate large amounts of solutions at a time. Kit is available through [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 in biohazardous waste box for incineration.

"The Green Bag®"

Another simple inexpensive 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 EtBr/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 back in the biohazardous waste box for incineration.

Green Bags are available through BIO101 (www.bio101.com) or through VWR (www.vwr.com) - this is a non-catalog item - use #BIO101 22350-200).

Finally, solutions containing heavy metals, organics, cyanides, sulfides, etc should be disposed as hazardous waste.

Gloves, Equipment and Debris

Gloves, test tubes, paper towels, etc. that are grossly contaminated with ethidium bromide should be placed in hazardous waste bags and labeled accordingly. If the items are significantly contaminated, consider deactivating in bleach before disposal.