



Guidelines for Ethidium Bromide Disposal

Ethidium bromide (EtBr) is commonly used as a marker for identifying and visualizing nucleic acid bands in electrophoreses and in other methods of gel-based nucleic acid separation. Although ethidium bromide is not regulated as a hazardous waste by the EPA, it is still a potent mutagen and is moderately toxic after an acute exposure and should *not* be discarded down the sewer drains with the exception of exempt quantities in exempt concentrations.

Ethidium Bromide Solutions

Only aqueous solutions containing ethidium bromide concentrations $<10 \mu\text{g/ml}$ (10 ppm) may be released to the sanitary sewer (drain disposal) with a one time approval from the EHS. Aqueous solutions containing $>10 \mu\text{g/ml}$ of ethidium bromide must either be collected, properly tagged, labeled and disposed of by means of our chemical hazardous waste vendor or treated using a charcoal filter (or other treatment method) and released through sanitary sewer disposal only once the filtrate solution's concentration has fallen below the permissible limits listed above.

[*\(click here for drain disposal guidance\)*](#)

Gels Containing Ethidium Bromide

Gels containing a concentration of ethidium bromide $<10 \mu\text{g/ml}$ (10 ppm) can be placed in a regular trash bag, tied and then placed in the regular laboratory trash for disposal. Gels containing ethidium bromide in concentrations $>10 \mu\text{g/ml}$ (10 ppm) must be placed in a large wide mouth plastic sealable container to prevent liquid from escaping. The container must remain closed unless adding gels and it must be properly tagged, labeled and disposed of through our chemical hazardous waste vendor.

Ethidium Bromide Contaminated Charcoal Filter

Charcoal filters used for the treatment of ethidium bromide solutions, once saturated and spent (follow manufacturer's instructions regarding recommended usage and disposal), can be collected in a large wide mouth plastic sealable container for disposal through means of our chemical hazardous waste vendor. The container must remain closed unless filters are being added and must be properly tagged and labeled at all times.

Glassware/Equipment Containing Ethidium Bromide

Glassware and equipment containing ethidium bromide should be emptied of the liquid solution containing the ethidium bromide and disposed of according to the procedures listed above. Contaminated glassware and equipment can then be triple rinsed and the rinsate can be released to the sewer system as long as the rinsate ethidium bromide concentration is $< 10\mu\text{g/ml}$ (10 ppm). Concentrations of rinsate greater than $10 \mu\text{g/ml}$ (10 ppm) must be disposed of according to the procedures listed above. [*\(click here for drain disposal guidance\)*](#)



Management of Ethidium Bromide Spills

EtBr spills can be decontaminated with a solution of 20 ml of hypophosphorus acid (50%) added to a solution of 4.2 g of sodium nitrate in 300 ml water. Prepare fresh solution the day of use in a fume hood. Wear rubber gloves, lab coat, and safety glasses. Turn off electrical equipment before decontamination.

1. Soak paper towel in decontamination solution, place on contaminated surface, and scrub.
2. Scrub five more times with paper towels soaked in water, using fresh towel each time.
3. Place all towels in a container and soak in fresh decontamination solution for one hour.
4. Test squeezings from final towel scrub and mixture for fluorescence; repeat procedure with fresh decontamination solution if fluorescence is present.
5. Neutralize with sodium bicarbonate and discard as nonhazardous aqueous waste.
6. This procedure has been validated for EtBr contaminated stainless steel, Formica, glass, vinyl floor tile surfaces, and filters of transilluminators.

Alternatives to Ethidium Bromide

Ethidium bromide (EtBr) is a dangerous compound due to its mutagenicity. SYBR Safe™ is a potentially safer alternative. Data on mutagenicity and EcoToxicity show [SYBR Safe™](#) is much less mutagenic than EtBr and is acceptable for discharge to the sanitary sewer. Several major institutions have switched from EtBr to SYBR Safe™ with

If you have any questions or need a waste determination performed please contact Robert Clay, Hazardous Materials Compliance Officer at 681-5497 or clayr2@lincolnu.edu



Various Ethidium Bromide Treatment Methods

Lunn and Sansone Method

For each 100 ml of ethidium bromide solution:

1. Add 20 ml 5% hypophosphorus acid.
2. Add 12 ml of 0.5 M sodium nitrate.
3. Stir briefly and let stand for 20 hours.
4. Adjust pH to 5-9 using sodium hydroxide or sodium bicarbonate.
5. Pour down drain and flush with copious amounts of water. ([click here for drain disposal guidance](#))

Armour Method

This is the simplest method, but is somewhat controversial involves bleach. One study found traces of mutagenic reaction mixtures using this method. Do not use this method.

Commercially available products, like "Destaining Bags" provide an alternative method of treatment for solutions. The destaining bags are simple to use and inexpensive. (Available through companies such as [GTS, Inc](#) and [Omega Bio-Tek](#)).

1. Drop a destaining bag into your solution,
2. Periodically swirl it around a few times,
3. Let it stand overnight.
4. In the morning, remove the bag and collect for disposal by the EHS.
5. Perform UV check of the solution. If it no longer fluoresces and no other hazardous chemicals are present, pour the solution down the drain. ([click here for drain disposal guidance](#))

EtBr Greenbag Kit

The EtBr GREENBAG™ Disposal Kit is a commercially-available product that consists of a "teabag" containing activated carbon which is placed into the waste solution to adsorb the ethidium bromide. The solution can then be disposed down the sanitary sewer, and the GREENBAG is to be subsequently disposed of as solid hazardous waste. This product is currently available from several suppliers, including [VWR](#) and [MP Biomedicals](#).



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. [Schleicher and Schuell](#) and [VWR](#) supply a commercial filter funnel kit that uses a packaged charcoal disk. This is particularly useful for labs that generate large amounts of solutions at a time. The kit is available through [Schleicher and Schuell](#) or [VWR](#).

1. Filter the ethidium bromide solution through the charcoal filter.
2. Pour filtrate down the drain. ([click here for drain disposal guidance](#))
3. Place charcoal filter in a sealed bag (e.g., zip-lock) and place in waste container for haz-waste pick-up.

The charcoal disks are graduated for easily tracking the amount of aqueous solution calculated for a fixed quantity of ethidium bromide residue (filtering up to ten liters per filter).

If you have any questions, need a waste determination, or a need a hazardous waste pick-up please contact Robert Clay, Hazardous Materials Compliance Officer at 681-5497 or clayr2@lincolnu.edu