



Ethidium bromide (EtBr) is a powerful mutagen widely used in biochemical research laboratories for visualizing nucleic acids. The compound forms fluorescent complexes by intercalation and these compounds are readily visible under ultraviolet (UV) light. EtBr is generally used in the laboratory dissolved in water or buffer solutions.

Since EtBr is so widely used, proper management of EtBr containing waste and EtBr contaminated materials is important. Below are the procedures to be followed for EtBr containing waste and EtBr contaminated materials:

Aqueous Solutions of Ethidium Bromide or Cesium Chloride/Ethidium Bromide

Aqueous solutions EtBr must first be treated before they are disposed of in the sanitary sewer or they may be sent to the OSU Chemical Waste Program. Aqueous solutions can be deactivated by the Lunn and Sansone method or by use of the AMRESKO destaining bags (concentrations <1.0 g/ml) and then disposed of in the sanitary sewer. Alternatively, the solution can be filtered through an EtBr filter. While wearing proper protective equipment, the paper carbon filter disk containing EtBr must be removed from the filter using a forceps and then processed through the Chemical Waste Program.

Acrylamide and Agarose Gels Containing Ethidium Bromide

Acrylamide and agarose gels containing EtBr must be processed through the OSU Chemical Waste Program.

Alcohol Solutions of Ethidium Bromide

Because alcohols are flammable, all alcohol solutions containing EtBr should be processed as hazardous waste through the Chemical Waste Program.

Labware Contaminated with Ethidium Bromide

Contaminated labware includes needles, disposable gloves, pipettes, test tubes, etc. that are contaminated with EtBr. Depending on the type of waste that you generate, follow the procedures below.

- Needles, scalpels, and other sharps contaminated with EtBr should be disposed of directly into a sharps container.
- Volumetric or transfer pipettes, and other disposable glassware contaminated with EtBr should be disposed of in a waste container designated for glass disposal. Grossly contaminated (visibly contaminated) glassware should be washed with bleach before disposal in the designated container.
- Test tubes and centrifuge tubes contaminated with EtBr should first be emptied, with the liquid disposed of according to the procedures given above. Empty tubes can then be disposed of in the trash. Grossly contaminated (visibly contaminated) tubes should be washed with bleach prior to disposal.
- Laboratory equipment (e.g. transilluminators, laboratory floors and countertops, etc.) contaminated with aqueous solutions of more than 10 mg/L EtBr should be decontaminated using the Lunn and Sansone method for decontamination of equipment contaminated with EtBr. This is done by scrubbing the contaminated lab equipment with paper towels soaked in a freshly prepared aqueous solution of sodium nitrite and hypophosphorous acid while wearing the proper protective equipment. The towels are then decontaminated in the decontamination solution before disposal in the trash.
- Most other disposable labware (e.g. sample vials, disposable beakers, etc.) contaminated with EtBr may be disposed of in the normal trash. Grossly contaminated (visibly contaminated) disposable labware should be washed with bleach prior to disposal.

Spills of Ethidium Bromide

Small spills (less than 0.5 L) of aqueous solutions of more than 10 mg/L EtBr and the resulting contaminated lab equipment can be decontaminated using the Lunn and Sansone method for decontamination of equipment contaminated with EtBr. The EtBr is degraded by wiping the surface with paper towels soaked in a freshly prepared decontamination solution (an aqueous solution of sodium nitrite and hypophosphorous

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acid) while wearing the proper protective equipment. All spills containing Ethidium Bromide should be reported to EHS at 541-737-2273.

Lunn and Sansone Ethidium Bromide Destruction Method

(Lunn, George, and Sansone, Eric B. 1994. *Destruction of Hazardous Chemicals in the Laboratory*. John Wiley and Sons, Inc. pp. 185.)

- Carry out the following steps in a fume hood and follow all laboratory safety precautions, including proper protective clothing.
- Dilute solutions containing EtBr to concentration <0.05% w/v (50mg/100mL).
- For each 100mL of EtBr solution add 20mL of fresh 5% hypophosphorous acid and 12mL of fresh 0.5M sodium nitrite solution. Check that the pH of the solution is <3.0. Stir briefly.
- After reacting for at least 20 hours, neutralize with sodium bicarbonate, then rinse the solution down the sanitary sewer with water.

Lunn and Sansone Method for the Decontamination of Equipment Contaminated with Ethidium Bromide

(Lunn, George, and Sansone, Eric B. 1994. *Destruction of Hazardous Chemicals in the Laboratory*. John Wiley and Sons, Inc. pp. 186.)

- Before decontamination, deenergize all electrical equipment and wear the appropriate protective equipment.
- The decontamination solution is prepared by adding 20mL of 50% hypophosphorous acid to a solution of 2g of sodium nitrite in 300mL of water.
- Scrub the contaminated surface or equipment with a paper towel soaked in the freshly prepared decontamination solution. Scrub another five times with paper towels soaked in the freshly prepared decontamination solution, using a fresh towel each time.
- Place all the used towels in a large container and soak them in fresh decontamination solution for at least one hour.
- Neutralize used decontamination solution and towels with sodium bicarbonate. The towels can then be discarded in the trash, and the solution may be rinsed down the sanitary sewer with water.

Note: A small amount of nitrogen dioxide may be given off when the decontamination solution is initially mixed, so the procedure should be carried out in a chemical fume hood.

AMRESKO Destaining Bags

- **Liquid ethidium bromide waste with a concentration less than 1.0 g/ml should be filtered via AMRESKO Destaining Bags (which look like standardtea bags).** The destaining bags are VERY SIMPLE and CHEAP to use. Simply drop a destaining bag into your solution, periodically swirl it around a few times, and let it stand overnight. In the morning, remove the bag and collect it as solid ethidium bromide waste. Then perform a spot check of the solution using a UV transilluminator to see if it fluoresces. If it does not, approximately 99% of the ethidium bromide has been removed and the solution is now safe to pour down the drain- provided no other hazardous chemicals are present.

Technical information from AMRESKO outlining the destaining bags capabilities and limitations can be obtained via their web at www.amresco-inc.com. (you will need to register with them to access the technical bulletin),

AMRESKO 800-829-2805