UIC The University of Illinois at Chicago
Environmental Health and Safety Office (MC 645)
1140 South Paulina Street
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Health-Safety@uic.edu

Chemical Waste Fact Sheet #7

Ethidium Bromide

In research laboratories, ethidium bromide (EtBr) and similar fluorescent compounds i.e., Acridine Orange, are used to visualize DNA on a gel. EtBr and its breakdown products are potent mutagens, suspect carcinogens and teratogens and at high concentrations, irritating to the eyes, skin, mucous membranes and upper respiratory tract. EtBr wastes are commonly collected as potentially hazardous waste by the UIC Environmental Health and Safety Office (EHSO). Although this waste is not regulated as a hazardous waste, we feel the mutagenic properties may present a hazard if disposed down the drain or in the regular trash.

Alternatives

Several commercial products that are considered safer5 and more environmentally sound have been developed as alternatives to EtBr.

MegaFluor fluorescent stain detection limit is reported to be in the low picograms (~3-5pg). There is no background in the gel because it is added to the sample, not the gel nor the buffer. As a result, it provides a consistent increase of exposure time when photographing the gel (up to 30 seconds) and thus the increased detection limit. With MegaFluor one can consistently differentiate between strands with just 2% difference in base pairs. More information can be found at http://www.euroclone.net.

SYBR Safe is less mutagenic, non genotoxic and non-hazardous for waste disposal. Details including a downloadable version of a report compiled by Molecular Probes, on the mutagenicity and environmental safety can be accessed at: http://probes.invitrogen.com/products/sybrsafe/ .

Contact 3-2436 for consultation on proper waste disposal.

Safety Precautions

Use "basic prudent practices" when handling EtBr. Because of its mutagenicity, stock solutions should be prepared in a properly functioning fume hood, (at least 6” from the edge) and protective nitrile gloves should be worn while handling this substance. Operations which may generate EtBr dust or aerosols of EtBr solutions should be conducted in a fume hood to prevent exposure by inhalation.

In the event of accidental skin contact, immediately wash with soap and water and remove contaminated clothing. In case of eye contact, promptly wash with copious amounts of water for 15 min (separating upper and lower lids and rolling eyeballs) and obtain medical attention at UIH Emergency Room. If EtBr is ingested, immediately proceed to the UIH Emergency Room. In the event of a spill, cover solid EtBr with a paper towel wetted with 10% bleach (avoid raising dust) for 10 minutes before collecting, with tongs, into an appropriate waste container. Wash area with bleach, followed by soap and water. Dispose solid materials in the waste container. Soak up aqueous solutions with a spill pillow or absorbent material, then wash with 10% bleach, followed by soap and water.

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Treatment

Recent findings of Margaret-Ann Armour of the University of Alberta show that dilute EtBr solutions can be safely treated with bleach to generate a non-mutagenic (according to the Ames Test) solution of 2-carboxybenzophenone. See "Tested Laboratory Disposal methods for Small Quantities of Hazardous Chemicals" in Waste Disposal in Academic Institutions, James Kaufman (editor), Lewis Publishers, 1990. The product is then suitable for disposal down the drain. We recommend the following procedure::

1. Carry out the following steps in a properly functioning fume hood, at least 6" from the edge.
2. For each 10 mg EtBr per 100 ml of solution, add 100 ml household bleach. (Bleach deteriorates over time upon exposure to air. If in doubt about the quality, use an excess and stir overnight.)
3. Stir at room temperature for 4 hours.
4. Rinse the destroyed EtBr solution down the sanitary sewer drain with 10 parts excess water.

Supelco (800-247-6628) provides a product that will filter EtBr waste to remove it from solution. However, the resin used to filter the waste must be disposed as hazardous waste. Other companies specializing in separation technologies may have similar products.

Disposal of Ethidium Bromide

Untreated solutions containing EtBr should be collected for disposal as potentially hazardous waste. Test tubes, gloves, papers, and other items may or may not require special treatment. If these items are clearly contaminated with EtBr, they should be disposed as hazardous waste. If they are not clearly contaminated, then they may be disposed in the normal trash. The laboratory user is usually the best person to determine reasonable disposal methods.

Trace amounts of EtBr in gels should not pose a hazard. Higher concentrations, e.g., when the color of the gel is dark pink or red, should not be placed in laboratory trash.

- Less than 0.1% ethidium bromide: place in laboratory trash
- More than or equal to 0.1%: place in watertight container for chemical waste pick-up.

Typically, campus researchers collect EtBr wastes and give them to the Health and Safety Section (HSS) for disposal. If you choose to dispose of these wastes through HSS, solids must be separated from liquids. Liquids, including those inside microcentrifuge tubes, should be emptied into poly containers with screw-cap tops. If you generate 2.5-5 gallons per month, you may request jerricans from HSS (3-2436). If you use a jerrican, EHSO will bring a clean one, if requested, when coming to collect your waste. Complete the information on the green label (provided) when you begin to fill the jerrican.

Test tubes and other glass or metal objects classified as sharps should be placed in the special sharps containers used for biohazardous waste. Other solids should be placed in plastic bags and labeled "Ethidium Bromide contaminated debris". For gels, use a wide-mouth poly jar with a screw cap top. Label jar "ETHIDIUM BROMIDE contaminated gel, HAZARDOUS WASTE, accumulated (date)". Do not overfill; leave a 2" air space. Waste pickups can be requested by using the Chemical Removal forms in the *UIC Hazardous Waste Management Guide* or http://www.uic.edu/depts/envh.