Safe Method of Use 16 – Disposal and Decontamination of Ethidium Bromide

Background

Ethidium bromide (EtBr; 2,7-diamino-10-ethyl-phenylphenanthridinium bromide) is widely employed for the rapid visualization of nucleic acids in electrophoretic gels. Though not a carcinogen, it behaves as a mutagen in the Ames Salmonella bioassay. It is widely used for visualizing nucleic acids. It may be harmful by inhalation, ingestion, and skin absorption and should be handled only when wearing nitrile gloves. If EtBr is to be weighed, the operation should be carried in a fume hood or a ventilated area.

Correct procedures for the disposal of EtBr depend on the nature of the waste materials and the concentration of EtBr that they contain. Much of the following advice is based on Maniatis et al. (1) and Lunn & Sansone (2, 3). Although Maniatis et al. offer a choice of protocols, the sodium nitrite method is preferred as the reaction products retain very little mutagenic activity. It is also relatively mild and so can be used to remove surface contamination. However, a small amount of nitrogen dioxide is given off when the components of the decontamination solution are mixed. Hence the procedure is best carried out in the fume hood.

Solid Waste

Small amounts of solid waste, such as tissues, gloves or stained electrophoresis gels, should be placed in appropriate packaging and sent for incineration. Although yellow bags can be used for this purpose, to avoid the possibility of leakage, yellow plastic bins are preferred for gels.

Contaminated sharps can be disposed of in sharps bins.

Bulk EtBr should be placed in a labelled container and disposed of via an approved waste contractor

Liquid Waste

A. Absorption Method

Dilute waste can also be decontaminated by absorption onto proprietary absorbents which include a column marketed by Merck that changes colour when exhausted, or the Green Bag marketed by Global Scientific. Waste can also be decontaminated by absorption onto Amberlite XAD-16 Persons wishing to use these alternatives should satisfy themselves that the products produce the desired effect when the manufacturers' instructions are followed.

Absorption on Amberlite XAD-16 Ion Exchange Resin – for solutions of EtBr < 0.1 mg/ml

- Dilute the aqueous ethidium bromide solution such that the total concentration of ethidium bromide does not exceed 0.1 mg/mL.
- For each 100 mL aliquot of ethidium bromide solution, add approximately 3.0 grams of Amberlite XAD-16 ion exchange resin and stir the resulting mixture for 20 hours.
- Filter the Amberlite resin from the aqueous solution and place it inside a yellow bag and send for incineration.

Activated Charcoal – for solutions of EtBr < 0.5 mg/ml

- Dilute to contain <0.5mg EtBr/ml - much liquid waste is already sufficiently dilute (e.g. electrophoresis buffer containing 0.5mg EtBr/ml).
- Add 100mg powdered active charcoal to each 100ml solution.
- Keep at room temperature for 1 hour, shaking intermittently.
- Filter through a Whatman No. 1 filter. Discard the filtrate.
- Wrap the filter and charcoal in a plastic bag. Place inside a yellow bag and send for incineration.

B. Bleach Decontamination Procedure (Ref 4) Armour Method

This procedure is somewhat more complicated and is therefore only recommended if extraction is not possible.

Perform the following in a fume hood:

- Dilute solutions of EB to a final concentration of less than or equal to 0.034% w/v (34 mg EB/100 ml solution).

- Add 10 ml fresh bleach for every 1 mg EB (bleach deteriorates upon exposure to air).

- Stir the mix continuously for 4 hours or overnight.

- Test the final solution with a UV light to ascertain that the EB is destroyed.

- Dispose final solution to sewer diluting 1 part solution with 20 parts tap water.

Please Note: this procedure is only for the decontamination of solutions containing ethidium bromide and not for surfaces as it relies on a 4 hour reaction time to ensure complete oxidation and conversion of all ethidium bromide to non-mutagenic 2-carboxybenzophenone.

C. Sodium Nitrite Procedure

This procedure is somewhat more complicated and is therefore only recommended if extraction is not possible.

- Dilute solutions with water to reduce the EtBr concentration to <0.5mg/ml.

- To the diluted solution, add 0.2 volume of fresh 5% hypophosphorous acid and 0.12 volume of fresh 0.5M sodium nitrite IN A FUME HOOD. Mix carefully. Important: check with indicator paper that the pH of the solution is <3.0 (if substantial amounts of buffers are present, it might be necessary to add more hypophosphorous acid. For mixtures containing alcohols, e.g isopropanol, 1-butanol, consult Ref No 2)

- Incubate 24 hours at room temperature. (A check for loss of fluorescence can be used to monitor completion of the inactivation process.) Add a large excess of 1M sodium bicarbonate before discarding.

NB:
Hypophosphorous Acid - is usually supplied as a 50% solution which is corrosive and must be handled with care. Dilute freshly before use.

Sodium Nitrite - dissolve 34.5g $NaNO_2$ in water and dilute to 1000ml. Note: there is a 2-fold discrepancy between the intended Molar concentration and the instructions for making up the solution. The present instructions accord with the original paper on EtBr inactivation by Lunn & Sansone (3)

Cleaning of Equipment and Laboratory Surfaces Contaminated with Ethidium Bromide

Glass, stainless steel, Formica, floor tiles, benches, fume hoods and the filters of transilluminators can be successfully decontaminated using the following technique. (No change in the optical properties of the transilluminator filter could be detected even after a number of treatments with the decontamination solution.)

- Unplug all electrical equipment before decontamination and wear appropriate protective equipment, including rubber gloves, lab coat, and chemical goggles.

- Make up the decontamination solution just prior to use.
  Dissolve 4.2 g of sodium nitrite in 250 ml water, IN A FUME HOOD slowly add 20 ml hypophosphorous acid (50%) and make up to a final volume of 300 ml with water.

- Wash the contaminated surface once with a paper towel soaked in the decontamination solution, taking care to avoid wetting electrical components. Then wash five times with water-soaked paper towels using a fresh towel each time.

- Soak all the towels in decontamination solution for 1 hour before disposal by incineration.

- Use a portable UV lamp to check that decontamination is complete. EtBr absorbs a broad range of UV light, so short (254nm), medium (300-315nm) or long (365-6nm) wavelength lamps can be used. Appropriate eye protection must be worn to guard the user against UV light while the lamp is switched on.

- Neutralize the used decontamination solution with sodium bicarbonate and discard as aqueous waste.

- Dry off the decontaminated surface or equipment. Arrange for electrical equipment to be checked by a competent electrician before plugging in for the first time unless you are absolutely certain that none of the electrical components have been wetted.

If the decontamination solution (pH 1.8) is considered to be too corrosive for the surface to be decontaminated, then use six H2O washes. Again, soak all towels in decontamination solution for 1 hour before disposal.

Emergency Exposure Procedures

- If EB contacts the eyes, immediately flush them with copious amounts of cold water for at least 15 minutes. (If it is available, an emergency eyewash is the best and safest way to do this.)

- For skin contact, immediately wash the affected area with soap and copious amounts of cold or cool water. If a person inhales EB dust, move him to an area where he can breathe fresh air. After any exposure to EB (via skin, inhalation, or eye contact), the affected person should immediately seek a medical attention.

References

1. Molecular Cloning, A Laboratory Manual; T. Maniatis, E.F. Fritsch & J. Sambrook (Cold Spring Harbor Laboratory, 2nd Edition, 1989, pages E8 – E9)
2. Destruction of Hazardous Chemicals in the Laboratory; G. Lunn & E.B. Sansone (Wiley Interscience, 1990, pp. 117-122, ISBN 0-471-51063-7).
3. G. Lunn & E.B. Sansone; Analytical Biochemistry, 1987, 162, 453-458.
4. Tested laboratory procedures for disposal of small quantities of hazardous chemicals in Waste Disposal in Academic Institutions, James A. Kaufman, ed., Lewis Publishers, 1990, p. 127.