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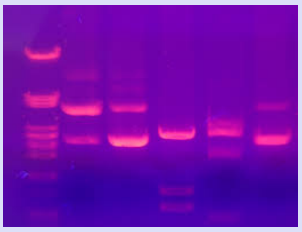
Safety Services Guidance



Guidance on the safe use and disposal of ethidium bromide

Key word(s):	Ethidium bromide, DNA stain
Target audience:	Lab users, Principal Investigators and Supervisors

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Introduction

1. This document is intended to provide specific guidance to assist with the decontamination of equipment contaminated with ethidium bromide (EtBr) and the disposal of material containing EtBr.

2. Ethidium bromide (EtBr; 2,7-diamino-10-ethyl-9-phenylphenanthridinium bromide) is a fluorescent dye widely used for the rapid visualisation of nucleic acids in electrophoretic gels. It is a potent mutagen and a possible carcinogen and/or teratogen. It is also toxic and so must be handled and disposed of correctly. Although EtBr is a very effective nucleic acid stain, its hazardous properties require rigorous control measures to be implemented to reduce associated risks; special waste disposal measures are also necessary. The Control of Substances Hazardous to Health (COSHH) Regulations require the use of hazardous substances to be avoided *so far as is reasonably practicable*. EtBr should only be used when product evaluation and risk assessment have shown that there is no appropriate safer alternative.

Safer Alternatives

3. The legal requirement is to substitute; where there is a safer product capable of achieving the same or similar results, this alternative must be adopted. Cost cannot be used as the sole reason for non-implementation of a less hazardous substance. A Safety Data Sheet (SDS) should be obtained for any product being considered as a substitute for EtBr, to allow a comprehensive evaluation of the product and its suitability to be carried out. Caution must be applied when making a substitution, to ensure that reducing exposure to a known hazard in the original substance does not inadvertently introduce another hazard of an equal or higher risk from the alternative substance.

4. Less mutagenic alternatives to EtBr are available. `SYBR Safe' and `MegaFluor' are two examples worth investigating.

Safe Working Practices

5. Refer to your risk assessment and Safety Data Sheet, and

    - always purchase ready-made stock solutions or tablets. The use of powders is strongly discouraged as it involves additional control measures eg face-fit testing, as respiratory personal protection will probably be required;
    - if weighing out cannot be avoided, use a fume cupboard or preferably an enclosed weighing station, wearing appropriate PPE (laboratory coat, gloves, goggles and mask);

- store stock solutions in dark bottles or bottles wrapped in aluminium foil at room temperature;
- design activities so as to minimise the potential for spills;
- avoid operations that are likely to generate dust or aerosols;
- when transporting EtBr-stained gels e.g. to the dark room, place the gels within a rigid box designated specifically for this purpose. Avoid contamination of door handles with EtBr by removing one glove and using the ungloved hand to open doors etc. Use similar techniques when operating other laboratory equipment and telephones, to avoid contaminating them with EtBr.

Disposal

6. Correct procedures for the disposal of EtBr will depend upon 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 Lune and Sansone[2]. 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 should be carried out in a fume cupboard. Treatment of EtBr with hypochlorite (bleach) is not recommended as the degradation products retain approximately 20% mutagenic activity (attributable to the presence of residual chlorine) in the Ames assay.

Solid Waste

7. Small amounts of solid waste, such as tissues, gloves or stained electrophoresis gels, should be placed in appropriate packaging and sent for incineration. Yellow plastic bins are preferable, to minimise leakage, but a yellow bag contained within a yellow plastic bin will suffice. Contaminated sharps can be disposed of in sharps bins. Bulk EtBr should be placed in a labelled container and disposed of via a licensed waste contractor.

Liquid Waste

8. Solutions containing EtBr should never be put down the sink without being decontaminated first. Decontamination systems should be located where the work is carried out to avoid unnecessary transport of buffers.

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[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); copy available in the Safety Reference section of the John Rylands University Library (catalogue No. 614.831 L20)

Dilute Liquid Waste (containing <0.5mg/ml, e.g. electrophoresisc) buffer containing 0.5µg/ml)

9. It is recommended that electrophoresis buffers and dilute solutions containing EtBr are decontaminated through a commercially available system. Two of the easiest and safest methods to use are the “Extractor” system from Sigma and Destaining bags.

10. Using the Extractor system, the EtBr is filtered through the extractor under vacuum. The filtrate can then be poured down the sink, providing there are no other environmentally hazardous components present. The filter can then be double-bagged and disposed of as solid waste for incineration once its capacity has been reached (10L of buffer containing 0.5ug/ml).

`Extractor' system (Sigma cat no. Z361569).

11. When using de-staining Bags (CLP cat no. 5459.25), EtBr gel tank buffer is poured into a container that has the required number of bags (see supplier’s information sheet). The ‘de-stained’ solution can then be put down the sink and the bags put in with the solid waste for incineration. It is advisable to double-bag these to prevent leakage.

De-staining Bags (CLP cat no. 5459.25),

12. Chemical treatments exist but are not recommended. EtBr-containing solutions can be decontaminated using activated charcoal as follows:

- 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 down the sink.
- Wrap the filter and charcoal in a plastic bag. Place inside a yellow bag within a solid waste bin and send for incineration.

13. Dilute waste can also be decontaminated by alternative absorbents. Persons wishing to use these alternatives should satisfy themselves that the products produce the desired effect when the manufacturers' instructions are followed.

Concentrated Liquid Waste (*containing > 0.5 mg/ml)*

14. Solutions containing > 0.5 mg/ml can be diluted to < 0.5mg/ml with water and then decontaminated using a commercial filter method as previously mentioned.

15. An alternative method is outlined below:

- 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** 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 Lunn and Sansone2).
- 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 down the sink.

* this is usually supplied as a 50% solution which is corrosive and must be handled with care. Dilute freshly before use.

** 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].

[3] G. Lunn & E.B. Sansone; Analytical Biochemistry, 1987, *162*, 453-458.

Cleaning of Equipment and Laboratory Surfaces Contaminated with EtBr

16. 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 goggles.
    - Make up the decontamination solution just prior to use. This consists of 4.2 g of sodium nitrite and 20 ml hypophosphorous acid (50%) in 300 ml H_2O. NOTE – the pH is approximately 1.8 so consider the corrosive effect on the surface to be decontaminated.
    - 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.
    - Dry off the decontaminated surface or equipment. Arrange for electrical equipment to be checked by a competent person before plugging in for the first time unless you are absolutely certain that none of the electrical components have been wetted.
    - 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. IMPORTANT - Appropriate eye/face visor protection (BS EN 170) must be worn to guard the user against UV light while the lamp is switched on.
    - Neutralise the used decontamination solution with sodium bicarbonate and discard as aqueous waste.

17. Commercially available products such EtBr Destroyer are also available. Their use should be risk assessed using the supplier's safety data sheet.

Personal Protective Equipment

18. The most common type of incident during use of EtBr gels is splashes to the eyes and face. These occur typically when withdrawing from the gels and looking closely at the markers. The risk assessment should specify all the PPE necessary, and this would normally include:

- Face Visors for UV - BS EN 170, selected for the specific wavelength used. Consult with the Radiological Safety Unit Technical Advisor.
- Gloves - nitrile gloves should provide adequate protection against skin contamination with EtBr. N-DEX gloves have been tested specifically against EtBr and the manufacturer claims they are three times more puncture resistant than thicker natural rubber gloves. Natural rubber latex gloves do not provide a suitable barrier to penetration by EtBr.
- Self-Monitoring for glove breakthrough - the simplest way to check for suspected contamination of the hands with EtBr is to place the operator's hands under a standard UV light (eye protection needed!). EtBr will show as a reddish/brown colour on the skin. Bench tops can be similarly monitored.

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