

Ethidium Bromide

Introduction

Ethidium bromide (EtBr) is widely used for visualization of nucleic acids in electrophoretic gels. EtBr forms fluorescent complexes, by intercalation of DNA, which are readily visible under ultraviolet (UV) light. EtBr is used either dissolved in an aqueous buffer solution and/or incorporated evenly throughout a gel. EtBr is available in powder, tablet or solution form.

Hazards

EtBr is strongly mutagenic. EtBr must also be considered a possible carcinogen and reproductive toxin. Therefore all individuals should regularly review their risk assessments and work practices for EtBr.

- Alternatives to EtBr must be used wherever possible.
- Pregnant workers should not work with EtBr.
- EtBr is readily absorbed through the skin.
- EtBr is highly toxic by inhalation (R26), particularly in powder form, and is irritating to the skin, eyes, mucous membranes and upper respiratory tract (R 36/37/38).
- The use of powdered EtBr is not recommended - pre-made solutions should be used.

Safety Precautions

Before using EtBr, you must do a thorough COSHH assessment and develop safe systems of work/ safe operating procedures. Assuming that 'safer' alternatives are not appropriate (see below) the first principle should be to minimise the quantity of EtBr used and to minimise the potential for cross contamination.

For example: typically no more than 5µl of a 10 mg/ml stock solution of EtBr is required for a 100 ml agarose gel (i.e. 0.5µg/ml), however reports indicate successful DNA staining with only 1µl (i.e.0.1µg/ml). Furthermore there is generally no need to add EtBr to running buffer. However the buffer will still become contaminated with EtBr from the gel as it runs. In addition, the following safety procedures must be observed:

- Always wear suitable, correctly fitting protective clothing when handling EtBr or EtBr waste i.e. fastened lab coat, safety glasses/goggles/face shield and chemically resistant gloves.

Beware that ordinary prescription glasses do not provide adequate protection. This is also true with poorly fitting safety glasses.

- Double gloving is advised. Direct handling, for example, using fingers to retrieve a gel from buffer should be avoided. Outer gloves should be changed frequently and after any contamination, whether deliberate or accidental.
- To prevent exposure by inhalation all experiments/procedures capable of generating EtBr dust or aerosols of EtBr must be performed in a fume cupboard, glove box or suitable chemical control facility, but not in a Microbiological Safety Cabinet.
- Always use a spill containment tray when working with EtBr. Areas where EtBr is used must be clearly defined (yellow/black tape).

- If you have to transport solutions of EtBr or EtBr-contaminated gels, use secondary container i.e. stock bottles in an outer liquid/shock proof plastic bottle, or gel trays in suitable plastic boxes etc.
- Reduce the risk of secondary contamination by putting on clean gloves after handling EtBr before touching any clean surfaces.
- Cleaning materials and waste facilities must be provided near to the working area and all equipment must be cleaned before and after use; including the surface of fume cupboards etc.
- Electrophoresis equipment i.e. gel tanks which are in 'continuous use' and therefore not decontaminated immediately after use must be strictly managed and properly labelled.

Storage

EtBr and EtBr stock solutions should be stored in a cool, dark, dry place separate from strong oxidising agents. Stock solution bottles should be of a type that is not easily knocked over and should be kept in a robust liquid proof secondary container when not in use. Do not store ETBR stock solutions at high level in the lab.

Disposal

EtBr waste includes:

1. Unwanted chemical stocks of EtBr/EtBr stock solutions
2. Solid waste (gels, contaminated paper towels, gloves etc)
3. Aqueous buffer solutions

1. Unwanted stocks/solutions of EtBr should be disposed of by Lab management – please contact us to arrange this. The original container should be used, but where this is not possible, it should be placed in a suitable, sound, leak-proof container and labelled clear with contents and centre.

2. Solid EtBr waste (e.g. gels, contaminated paper towels, plastic-ware, and gloves) should be placed into a clearly labelled, suitable, leak-proof container (e.g. lidded bucket) which is lined with an appropriate polythene bag. Full bags should be sealed, removed from the container and placed inside a second robust polythene bag, for disposal. Loose needles and syringes must be disposed of in a 'sharps' container.

3. Aqueous buffer solutions may be disposed of in any of the following ways:

- The buffer can be collected in a suitable container and disposed of as Hazardous Chemical Waste (as above).
- The EtBr can be removed using a de-staining 'tea-bag'. Several makes of de-staining bag are available including self-indicating de-stain bags. The manufacturer's instructions should be followed but in general, use 1 'tea-bag' per 10mg of EtBr (max). Leave stirring for 24 hours. The 'tea-bag' should be discarded as in (2) above. The remaining solution can be discarded to drain.
- The EtBr can be adsorbed onto an ion-exchange column specifically for this purpose. The waste liquid may then be discarded to drain. The expended columns should be double-bagged (polythene) and disposed of as in (2) above.

- The EtBr may be adsorbed onto activated charcoal at a rate of 100mg charcoal to 50mg EtBr. The mixture should be left stirring overnight before filtering off the solid for disposal as in (2) above.

The liquid filtrate can then be discarded to a drain.

Disposal of EtBr down the sink or drains is strictly prohibited.

Emergency Procedures

EtBr Spills

Appropriate personal protective equipment (PPE) must be worn when dealing with spillages. Spills of EtBr solution should be immediately absorbed onto a neutral absorbent material e.g. paper towels, vermiculite or absorbent granules and the area decontaminated (see below). All spill clean-up materials and absorbents should be double-bagged in polythene bags or placed in a sealed container and passed on to lab management to be disposed of as hazardous chemical waste.

If necessary use a hand-held UV lamp to check for residual EtBr contamination following a clean-up. A reddish-orange fluorescence can be detected under both 'long' and 'short' UV wavelengths. Remember that there are additional hazards associated with the use of UV, all persons must wear suitable PPE to cover the skin and eyes (close fitting lab coats, long cuffed gloves and UV face shields).

Decontamination

For small spills of weak concentration e.g. up to 10 ml of 10 mg/ml EtBr – after mopping up the spill, wash the area down with a 50:50 mixture of isopropyl alcohol and water. All contaminated liquids and solids should be passed on to lab management for disposal as hazardous chemical waste.

There are now many commercial pre-mixed EtBr decontamination products which are available as a spray and are preferable to using lab chemicals as the hazards associated with mixing solutions containing acid are eliminated.

The following solution can also be used to decontaminate equipment and areas and could be of particular use after a serious spillage or for a difficult decontamination. This decontamination solution must be prepared immediately prior to use.

- Mix 20ml of hypophosphorus acid (50%) (H_3PO_2) to a solution of 4.2g sodium nitrite ($NaNO_2$) in 300ml water. Prepare this solution in a fume cupboard as a small amount of nitrogen dioxide may be given off when the solution is initially mixed. Care should be taken due to the acidity of the solution (pH 1.8).

Decontamination procedure:

1. Wash the contaminated surface or equipment once with a paper towel soaked in freshly prepared decontamination solution.
2. Wash the area/equipment 5 times with paper towels soaked in tap water, using a fresh towel each time.*

3. Using a UV light, check to ensure that all the EtBr has been removed (absence of reddish-orange fluorescence) – use appropriate precautions and PPE.

4. Soak all towels in the decontamination solution for 1 hour.

5. Neutralise used decontamination solution and towels with sodium bicarbonate.

6. Discard the towels in the general waste and rinse the solution to normal drain with copious amounts of water.

* If the acidic nature of the decontamination solution is capable of damaging the contaminated surface, use additional rinses.

2. Accidental Exposure to EtBr

If you are wearing the correct protective clothing and following procedures, this is unlikely to occur, however:

- In case of eye contact, immediately flush eyes with copious amounts of water for at least 15 minutes. Seek medical advice.
- In case of skin contact, immediately wash the affected area with soap and copious amounts of cold or cool water and remove contaminated clothing. Seek medical advice.
- If ingested or inhaled, remove to fresh air. Seek medical advice immediately.
- Report it via an Accident Report Form.

EtBr Alternatives

Continual risk assessment requires that if any hazardous procedure or chemical can be substituted with a safer alternative it must be done – regardless of cost.

Several alternatives to EtBr exist which manufacturer's claim (but do not fully substantiate) are less toxic, can detect nucleic acid components at lower concentrations than EtBr and may not require UV light sources.

Examples include 'Redsafe', 'GelRed', 'SYBR Green', 'SYBR Safe' and 'Megafluor'.

Laboratory tests have concluded that these stains work as well as, and in some cases better than EtBr.

Toxicological information on these products is limited. However, since they work in a similar way to EtBr the same control measures used for handling of EtBr must be followed. All DNA stains must be treated as hazardous chemical waste and not allowed to go down sinks or drains.

See manufacturer's advice on handling and decontamination.

EtBr Key Points

- There is no acceptable level of contamination.
- Always buy EtBr as ready-made solutions of required strength
- Minimise the amount used and only buy what you need

- Work in a designated labelled area using benchkote to minimise/mitigate the effects of potential contamination
- Wear 2 pairs of long cuffed disposable nitrile gloves when handling EtBr plus full face/eye protection and a fastened laboratory coat which is correctly fitted to cover exposed skin
- Dispose of EtBr containing gels as hazardous chemical waste; double bagged and labelled
- Use self-indicating de-stain bags for treating used and surplus buffer solutions, or dispose of as hazardous chemical waste