

Chemical Safety Guidance Series

February 2020

Ethidium Bromide User Guidance

Occupational Health and Safety Service
HSD123C (rev 4)



**UNIVERSITY OF
CAMBRIDGE**

Guidance for Using Ethidium Bromide

This leaflet provides you with the information you need for assessing the risks of using ethidium bromide and for developing a safe operating procedure (SOP).

Uses:

Ethidium bromide (EtBr) is widely used for rapid 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 **or** incorporated evenly throughout a gel matrix.

EtBr is available in powder, tablet or solution forms, however, the hazards associated with handling the powder should wherever possible be avoided by purchasing tablets or pre-made solutions.

Hazards:

The International Agency for Research on Cancer (IARC) does not currently class EtBr as either a possible, probable, or confirmed human carcinogen.

However, **EtBr is a category 2 mutagen**, that is, a substance which may induce heritable mutations, determined by the in-vitro Ames test, therefore **pregnant workers should avoid working with EtBr, consider using an alternative, see below.**

EtBr is highly toxic by inhalation (R26 – H330), therefore the use of powdered EtBr is NOT recommended.

EtBr is also irritating to the skin, eyes, mucous membranes and upper respiratory tract (R 36/37/38 – H 319/335/315),

EtBr is readily absorbed through the skin.

Alternatives to EtBr:

Several alternative DNA stains are claimed by manufacturer's to be, less toxic, to be able to detect nucleic acid components at lower concentrations and in some cases may not require UV light sources. Examples include, but are not limited to, GelRed, GelGreen, SYBR Green, SYBR Safe etc. Toxicological information on these products is limited beyond the Ames test, however, since by definition they interact with DNA it would be prudent to use the same control measures as EtBr. See manufacturer's advice for information on handling and decontamination of these stains.

HOWEVER: In accordance with the wishes of the Anglian Water Company: ALL solutions containing ethidium bromide must NOT be allowed to go down sinks or drains untreated, but should be pre-treated to remove the stain before disposal of the decontaminated liquid.

Safety Precautions:

In accordance with the COSHH regulations, the first principle should be to minimise the quantity of EtBr in use. No more than 5µl of a 10 mg/ml stock solution of EtBr is needed for a 100 ml agarose gel and for many purposes as little as 1µl is sufficient. To put this in perspective, this represents dilution factors of 1 in 20,000 to 1 in 100,000 of the 10 mg/ml stock solution.

There is generally **no need to add EtBr to running buffer**, a process that only increases potential contamination and exposure, but it must be remembered that even without deliberate addition the buffer will still become slightly contaminated by EtBr migrating from the gel as it runs.

In addition, the following safety precautions should be observed:

- **Wherever possible, purchase ready-made EtBr stock solutions.**
- 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:
 - **Ordinary prescription glasses and poorly fitting safety glasses do not provide adequate protection as splashes can get under, over or around the lenses.**
 - Only good quality gloves with the best available Acceptable Quality Level (AQL 0.65) and a known chemical permeation breakthrough time for EtBr should be used. For example Kimberly Clark 'Kimtech' purple nitrile gloves have an AQL of 0.65 and a chemical breakthrough time in excess of eight hours for EtBr.
 - Natural latex disposable gloves do NOT provide adequate protection.
- Dipping fingers into buffer to retrieve a gel should be avoided. However if absolutely necessary, then double gloving (wearing two pairs of disposable gloves, one pair over the other) is advised as disposable gloves can be manufactured with pin holes in some of them (see AQL data). The outer gloves should be changed 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.
- Reduce the risk of secondary contamination by removing EtBr contaminated gloves, replacing with fresh gloves where necessary after handling EtBr and before touching any clean surfaces.
- Do NOT wear contaminated gloves when opening doors or handling equipment / apparatus.
- Always use a spill containment tray when working with EtBr. It is best to have a clearly defined area of the lab for running gels covered with fresh benchkote or similar absorbent material which is changed regularly and whenever grossly contaminated.
- Transport stock solutions of EtBr in a robust shock and spill proof secondary container,
- Transport wet gels / wet gel trays in an appropriate secondary container, such as a picnic box, to prevent drips, particularly when moving between laboratories / areas.
- 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 e.g. nitric acid. Stock solution bottles should be of a type that are **not** easily knocked over and should be kept in a robust shock and spill proof secondary container when not in use. As with all chemicals, containers should be kept tightly closed and unauthorised access prevented.

Waste disposal:

EtBr waste streams typically include:

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 must be disposed of via the hazardous chemical waste disposal service. A service which is run centrally through the Occupational Health and Safety Service. 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 appropriately following departmental procedures.
2.
 - a. Mixed solid EtBr waste (e.g. gels, low level contaminated paper towels, plastic-ware, and gloves) may be disposed of in the general waste stream for land fill (not recycling) in robust plastic bags (double bagging may be required).
 - b. Alternatively, **solid waste consisting solely of contaminated gels** may be placed in a clearly labelled, suitable, leak-proof container (e.g. lidded bucket) which is lined with an appropriate polythene bag. Although probably still below the legal threshold for hazardous waste (see University guidance) they can be disposed of via the hazardous chemical waste disposal service.

EtBr gels containing radioisotopes must be disposed of as solid radioactive waste.

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:
 - a. **The preferred method is to decontaminate the buffer before disposal to drain.** EtBr/stain can be removed using a de-staining 'tea-bag'. Several makes of de-staining bag are available including self-indicating destain bags. The manufacturer's instructions should be followed but in general, use one 'tea-bag' per 10mg of EtBr (max). Leave stirring for 24 hours. The 'tea-bag' should be discarded as hazardous chemical waste. **The remaining de-contaminated solution can be discarded to drain.**
 - b. 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 hazardous chemical waste.
 - c. 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 hazardous chemical waste. The liquid filtrate being discarded to drain.
 - d. Alternatively, the buffer can be collected in a suitable container and disposed of via the hazardous chemical waste disposal service; however this will incur a charge to the Department / Institution.

Disposal of EtBr or other DNA stains directly down the sink or drains is strictly prohibited.

Emergency Procedures:

1. Spillages

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 X-sorb and the area decontaminated (see below).

Avoid raising dust when cleaning up solid spills by gently mixing with water and then absorbing the solution as above.

All spill clean-up materials and absorbents should be double-bagged in polythene bags or placed in a sealed container and if heavily contaminated should be disposed of as hazardous chemical waste as per the department's procedures.

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.

Decontamination

For small spills e.g. up to 10 ml of 10 mg/ml stock EtBr solution –

- Mop up the spill with an absorbent paper towel and place the contaminated towel(s) directly into a plastic bag and seal to avoid cross contamination, treat this as Hazardous Waste.
- Wipe the area down with a 50:50 mixture of isopropyl alcohol (IPA) and water to remove as much residual stain as possible.
- Any remaining surface staining can be carefully removed with a small amount of dilute bleach, followed by rinsing with water. **However**, bleach must **not** be used directly on solid or liquid spills as this produces a highly toxic product.
- Dispose of any highly contaminated liquids and solids used in the clean-up procedure as hazardous chemical waste.

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 (see below), check to ensure that all the EtBr has been removed (absence of reddish-orange fluorescence).
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 exposure via the University Accident Report Form (HSD020E - Safety Office website).

Sources of Ultra Violet Light:

Remember that there are additional hazards associated with the use of Ultra Violet light (UV), **all** persons must wear suitable PPE to cover the skin and eyes (close fitting lab coats, long cuffed gloves and UV face shields), see University Guidance on the safety office website.

Note: UV face shields provide adequate protection for normal exposure times when working with a trans-illuminator however for extended periods of exposure, consult the manufacturer's data.

Further Information

The following Occupational Health and Safety Service publications are available on Safety Office website:

- Working Safely with Artificial Sources of Ultraviolet Radiation
- Ultraviolet Radiation in Biological Applications
- Guide to Hazardous Substances Risk Assessment & RA Form
- Working Safely with Carcinogens, Mutagens and Substances Toxic to Reproduction
- Health Record Form
- Hazardous Substances Policy
- Safe Chemical Practice
- Disposal of Chemical Waste: Policy and Guidance

EtBr Key Points

Handle carefully as there is no acceptable level of contamination.

Whenever possible buy EtBr as a ready-made solution.

Minimise the amount used and only buy what you need.

Work in a designated labelled area using Benchkote to minimise the effects of potential contamination.

Wear 2 pairs of long cuffed disposable nitrile gloves, close fitting safety glasses and a fastened laboratory coat which is correctly fitted to cover exposed skin when handling EtBr .

Double bag EtBr containing gels before disposal.

Use self-indicating destain bags for treating used and surplus EtBr containing buffer solutions.

History

Reviewed in 2020 as rev 4 with only very minor changes



Safety Office
Greenwich House
Maddingley Road
Cambridge
CB3 0TX

Tel: 01223 333301
Fax: 01223 330256
safety@admin.cam.ac.uk
www.safety.admin.cam.ac.uk/

HSD123C (rev 4)
© University of Cambridge