

STANDARD OPERATING PROCEDURE ETHIDIUM BROMIDE USE AND DISPOSAL

Introduction

Although ethidium bromide (EtBr) is not regulated as a hazardous waste, its mutagenic properties may present a human health hazard if it is placed in the trash or poured down the sanitary sewer system. Use the following procedures when working with or disposing EtBr solutions, gels, or other EtBr contaminated materials.

Safe Handling

Store EtBr and EtBr stock solutions away from strong oxidizing agents (e.g. nitric acid) in a cool, dark, dry place. Stock solution bottles should be kept in a robust liquid proof secondary container when not in use. Make sure that the stock solution bottles are of a type that is not easily knocked over. As with all chemicals, containers of EtBr should be kept tightly closed.

All procedures involving pure EtBr, concentrated EtBr stock solutions, or EtBr gels should be performed in a chemical fume hood.

For incidental contact with EtBr (minimum potential for splash and exposure), wear safety glasses with side shields and brow guard protection or goggles, double nitrile gloves, and a non-porous lab coat. If your outer gloves become contaminated, replace them immediately with clean ones. Natural rubber latex gloves do not provide a suitable barrier to EtBr penetration.

For prolonged contact, work involving concentrated solutions, spill cleanup, or large quantities of EtBr, wear chemical splash goggles, double nitrile gloves, and an impervious chemical-resistant or polyvinyl chloride apron/smock/lab coat. Avoid using the traditional cotton-polyester white lab coat that readily collects/absorbs compounds. If your outer gloves become contaminated with EtBr, replace them immediately with clean ones. Also be sure to remove your gloves when handling non-lab items such as telephones or keyboards.

EtBr should only be added to molten agarose solutions when the latter have been allowed to cool below 50°C (122°F). This prevents the possible release of EtBr in vapor form.

When transporting EtBr-stained gels (for example, to the dark room), use a rigid box to contain the gels. Designate a box specifically for this purpose. You must avoid contamination of door handles with EtBr by using an ungloved hand to open doors ([SOP: Proper Use of Gloves](#)).

How to Protect Yourself When Viewing Electrophoretic Gels

Short wave ultraviolet (UV) radiation will harm your eyes and skin. *Ordinary prescription eyeglasses do not protect your eyes from this intense UV.* When using UV light to visualize EtBr, you must wear UV-blocking eyewear, full-face shield, long-sleeve protective clothing, and gloves for protection.

Disposal

Disposal of EtBr solutions into the sanitary sewer or sink drain is not permitted. EtBr gels and

unwanted solid EtBr must be disposed through Environmental Health and Safety Office (EH&S). Liquids or working solutions may also be disposed through EH&S or rendered non-toxic by the user. Gels may be accumulated in plastic jars or buckets which are lined with an appropriate polythene bags and have lids. These containers must be clearly marked with words “Hazardous Chemical Waste” and “EtBr, Mutagenic”. Full bags should be sealed, removed from the container and placed inside a second robust polythene bag for disposal. Do not mix other waste with gels such as plastic wrap, gloves, paper towels, sharps, or other contaminated items. These solid materials must be packaged separately and labeled as chemically contaminated items. Sharps must be deposited in a puncture resistant container and labeled appropriately. Label all waste with a Hazardous Chemical Waste tag and make a request for disposal via [Chemical Environmental Management System \(CEMS\)](#). Follow the instructions in the [Standard Operating Procedure on how to request for chemical waste removal on CEMS](#).

Decontamination

For small spills of weak concentration (e.g., up to 10 ml of 10 mg/ml EtBr) after wiping up the spill with paper towels, wash the area down with a 50:50 mixture of isopropyl alcohol (IPA) and water. Dispose of all contaminated solids as hazardous chemical waste.

If you spill a large quantity of EtBr, immediately decontaminate the area following the procedure below:

Prepare this decontamination solution just prior to use:

- 4.2 g sodium nitrite (NaNO_2 , CAS No. 7362-00-0)
- 20 mL 50% hypophosphorous acid (H_3PO_2 , CAS No. 6303-21-5)
- 300 mL water

Wearing full Personal Protective Equipment (eye protection, gloves and apron/smock/ lab coat) wash the area with paper towels soaked in decontamination solution. Rinse the area five times with paper towels soaked in tap water, using a fresh towel each time.

1. Using a UV light and wearing eye protection check the area to ensure that you have removed all EtBr. Repeat decontamination procedure as necessary. If the acid could damage the contaminated surface, use a few additional rinses with paper towels soaked in tap water.
2. Soak all the towels in decontamination solution for one hour. Then remove the towels, gently wring out any excess solution back into the decontamination container and dispose of the towels as dry hazardous waste in a doubled bag along with the contaminated gloves.
3. Include the decontamination solution and all solids in your hazardous waste pickup.
4. Inform your Principal Investigator (PI).

Note: Do not use bleach to cleanup EtBr spills. The combination may be explosive.

Alternatives to EtBr

Consider switching to less-toxic alternatives (for example, SYBR Safe™ DNA gel stain, GelRed™, GelGreen™, MegaFluor™) to EtBr to reduce potential hazardous exposures in the laboratory. Disposal of most EtBr alternatives must be managed in a manner like that described above for EtBr.

References

en.wikipedia.org/wiki/Ethidium_bromide

Revised 8/2/2023