

STANDARD OPERATING PROCEDURE:  
ETHIDIUM BROMIDE (EtBr) - CAS No. 1239-45-8

PI: \_\_\_\_\_ Room & Building: \_\_\_\_\_  
Department: \_\_\_\_\_ Research Group: \_\_\_\_\_  
Date: \_\_\_\_\_ Pertains to Lab Protocol: \_\_\_\_\_

### USE & PROCEDURE

Attach the experimental protocol(s) that involve the use of ethidium bromide (EtBr).

### POTENTIAL HAZARDS

- Potent mutagen (may cause genetic damage)
- Acutely toxic
- Irritant to the skin, eyes, mucous membranes, and upper respiratory tract

### ENGINEERING/VENTILATION CONTROLS

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

### RECOMMENDED PERSONAL PROTECTIVE EQUIPMENT

The level of skin and eye protection should be selected based on the potential for splashing and other forms of exposure.

*Incidental contact (minimum potential for splash & exposure):*

- ✓ Safety glasses with side shields and brow guard protection
- ✓ Double nitrile gloves
  - Change outer gloves frequently to minimize cross-contamination.
  - Immediately replace with new gloves when splash occurs.
- ✓ Protective clothing (e.g. non-porous lab coat, closed-toed impervious footwear)

*When handling pure EtBr, concentrated stock solutions, large quantities, or for spill clean-up:*

- ✓ Chemical splash goggles (if not working in a fume hood or if the hood's sash is not down)
- ✓ Double nitrile gloves
  - Change outer gloves frequently to minimize cross-contamination.
  - Immediately replace with new gloves when splash occurs.
- ✓ Impervious chemical resistant apron/smock/lab coat (PE or PVC)
  - Avoid using the traditional cotton-polyester white lab coat, which readily collects/absorbs compounds.
- ✓ Closed-toed, non-porous footwear

**Note:** Natural rubber latex gloves do not provide a suitable barrier to penetration by EtBr.

## ADDITIONAL PRECAUTIONS

- EtBr should only be added to molten agarose solutions when the latter has been allowed to cool below 50°C. This prevents the possible release of EtBr in the vapor form.
- When transporting EtBr-stained gels (e.g. to the dark room), the gels must be contained within a rigid box designated specifically for this purpose. The operator must avoid contamination of door handles with EtBr by using an ungloved hand to open doors.
- Short wave ultraviolet radiation will harm your eyes and skin. When using ultraviolet light to visualize EtBr, the user should wear UV-blocking eyewear, full-face shield, long-sleeve protective clothing and gloves for protection.
- EtBr should be stored away from strong oxidizing agents in a cool, dry place and the container must be kept undamaged and tightly closed.

## MATERIAL SAFETY DATA SHEETS

MSDSs are available electronically via EHS Department's Web page:

<http://www.uos.harvard.edu/ehs/msds/>. An option, but consider collecting in a binder the MSDSs that arrive with each order.

## DECONTAMINATION PROCEDURE<sup>1</sup>

Surface decontamination should be performed using the following solution:

- 4.2 g of sodium nitrate (NaNO<sub>2</sub>, CAS No. 7362-00-0)
- 20 ml of 50% hypophosphorous acid (H<sub>3</sub>PO<sub>2</sub>, CAS No. 6303-21-5)
- 300 ml of water

The following procedure should be followed to perform decontamination:

1. Wash the area with a paper towel soaked in decontamination solution. Rinse the area five times with paper towels soaked in tap water, using a fresh towel each time.
2. Using a UV light, check the area to ensure that all the ethidium bromide has been removed. 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.
3. 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 waste in a doubled bag along with the contaminated gloves.
4. Contact EHS (432-1720) for guidance on disposing of the decontaminated solution, towels, and gloves used in the cleanup or refer to the online "Ethidium Bromide Fact Sheet" provided by Harvard EHS ([http://www.uos.harvard.edu/ehs/environmental/ethidium\\_bromide.shtml](http://www.uos.harvard.edu/ehs/environmental/ethidium_bromide.shtml)).

**Note:** Decontamination solution should be prepared just prior to use. Full protective equipment (as described in "Recommended Personal Protective Equipment" above) should be worn when preparing or using the decontamination solution.

## WASTE

Refer to the *Laboratory Waste Guide* posted at

<http://www.uos.harvard.edu/ehs/longwood/HarvardLongwoodLabWasteGuide.pdf>

- All EtBr wastes (liquid, solid and debris) must be managed as hazardous chemical waste.
- See *Appendix A* below (also available at [http://www.uos.harvard.edu/ehs/environmental/ethidium\\_bromide.shtml](http://www.uos.harvard.edu/ehs/environmental/ethidium_bromide.shtml)) for more details.

## ETHIDIUM BROMIDE ALTERNATIVES

Consider switching to less-toxic alternatives (ex. SYBR Safe™ DNA gel stain, GelRed™, GelGreen™, MegaFlour™) to EtBr to reduce potential hazardous exposures in the lab.

- Disposal of most EtBr alternatives must be managed in a manner similar to that described above for ethidium bromide.
  - *Exception:* SYBR Safe™ DNA gel stain has been approved by the Massachusetts Water Resources Authority to be disposed of by sewer.
- Contact EHS (ext. 2-1720) for specific guidance if you have any questions regarding proper disposal of EtBr alternatives.

## EMEGENCY PROCEDURES

Refer to the emergency flip chart titled “*EHS Procedures and Response Guidelines*,” posted in each laboratory and found on EHS’s webpage at <http://www.uos.harvard.edu/ehs/longwood/>

- All spills of EtBr should be cleaned up immediately using the surface decontamination procedure listed above.
- Do not use bleach solutions to clean up EtBr contamination. It is not effective and can be explosive under certain conditions.

### *References:*

1. Lunn, G. & Sansone, E.B., *Destruction of Hazardous Chemicals in the Laboratory*, John Wiley & Sons, New York, 1990, p. 119-120.

## APPENDIX A: ETHIDIUM BROMIDE WASTE HANDLING

Waste Stream	Description	Waste Management Procedure
<b>Buffer Solutions</b>	Typically contain very small concentrations of EtBr (<0.01% by wt.).	<p>May be discharged to sinks.</p> <p>Consider removing EtBr from waste solution prior to discharge to sink by using commercially available cartridges.</p> <p>Spent cartridges must be collected in waste containers designated for this purpose.</p> <p>Keep waste containers in secondary containment bins, closed when not in use, and labeled as “Hazardous Waste: Ethidium Bromide (Mutagen)”. When labeling, be sure to check the “Other” box under hazard type and specify “Ethidium Bromide (Mutagen)”.</p> <p>Store waste containers at or near hazardous waste satellite accumulation area.</p> <p>Contact EHS for assistance or waste removal (432-1720).</p>
<b>Gels</b>	Typically contain small amounts of EtBr.	<p>Collect in waste containers/bags designated for this purpose. Double-bag (if bags are used).</p> <p>Keep waste containers/bags in secondary containment bins, closed when not in use, and labeled as “Hazardous Waste: Ethidium Bromide (Mutagen)”. When labeling, be sure to check the “Other” box under hazard type and specify “Ethidium Bromide (Mutagen)”.</p> <p>Collect waste gels in 5 gallon twist-off lid bucket lined with clear 4 mil poly bags. Please DO NOT use biohazard bags or place gels into bucket without liner. Both buckets and liners are available from EHS. For supplies and waste removal, call 432-1720.</p>

