Lab Standard Operating Procedure:

**Ethidium Bromide (EtBr) - CAS No.** **1239-45-8**

PI: Room & Building:

Department: Research Group:

Date: Pertains to Lab Protocol:

**PROCEDURE**

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

**POTENTIAL HAZARDS**

* Mutagen (may cause genetic damage) at high concentrations (stock solution or powders)
* Toxic when working with stock solutions, powder or crystals.
* **Avoid working with Ethidium Bromide in the raw form** (powder or crystal). Order pre-diluted stock solutions.
* Irritant to the skin, eyes, mucous membranes, and upper respiratory tract when working with stock solutions and powders.

**ENGINEERING/VENTILATION CONTROLS**

All procedures involving Ethidium Bromide powder *must* be performed in a fume hood or effective local exhaust. Stock solutions of Ethidium Bromide are best handled in a fume hood or at a local exhaust.

**PERSONAL PROTECTIVE EQUIPMENT**

(Refer to your lab’s PPE Assessment Report, supplemented with information here)

*Powder Use must be in fume hood or effective local exhaust:*

* Safety glasses *required*
* Double layers of nitrile gloves *required*
  + Change outer gloves frequently to minimize cross-contamination.
  + Immediately replace with new gloves when splash occurs.
* Lab coat *required*

*Pipetting from Stock Solutions (Container size of 10mg/ml stock solution must be < 20 ml):*

* Local ventilation or fume hood recommended
* Safety glasses required
* Nitrile gloves required
  + Immediately replace with new gloves when splash occurs.
* Lab coat strongly recommended when transferring from stock solutions.
* Closed-toed footwear required when working with chemicals

*Removing hot solutions from microwaves:*

* Safety glasses and lab coat strongly recommended
* Heat Resistant gloves recommended

*Other operations:*

* Recommend wearing safety glasses and gloves when handling gels and buffer solutions.
* 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.

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

**ADDITIONAL PRECAUTIONS**

EtBr stock solutions and powder should be stored away from strong oxidizing agents in a cool, dry place and the container must be kept undamaged and tightly closed.

**DECONTAMINATION PROCEDURE**

***Stock* Solution Spills**

Surface cleanup should be performed using the following solution:

* Simple Green/detergent and abrasive pads such as steel wool or brillo pads

Use the following procedure for surface cleanup:

1. Put on heavy duty gloves (preferred, since abrasive pad may puncture thin nitrile gloves) or double layers of nitrile gloves.
2. Soak the spill up with paper towels or some other absorbent and place absorbents in a secondary container that does not leak.
3. Once the area is dry spray the affected area with Simple Green and then scrub the area with a steel wool or brillo pad for several minutes.
4. Using paper towels dry up the area and then wipe the area down with absorbents dipped in tap water. Repeat this process until the area is clean.
5. Using a UV light, check the area to ensure that all the ethidium bromide has been removed. Repeat decontamination procedure as necessary.
6. Place all contaminated towels, pads and other debris in a secondary container (non-leaking) with a lid. Label as "Hazardous Waste, Ethidium Bromide (Mutagen)". When labeling, be sure to check the "Other" box under hazard type and specify "Ethidium Bromide (Mutagen)".Contact EHS at 496-3979 (Cambridge) or 432-1720 (Longwood) for guidance on disposing of the decontaminated solution, towels, and gloves used in the cleanup.

**Powder or Crystal Spills**

Use wet paper towels (soaked in Simple Green/detergent or water) and place over the contaminated area and then place towels in hazardous waste bag or a sealed mayonnaise jar.

Once this is completed follow steps 1-7 above.

**Note:** Full protective equipment (as described in “Recommended Personal Protective Equipment” above) should be worn when cleaning up spills of stock solution, powder or crystals.

**ETHIDIUM BROMIDE ALTERNATIVES**

Consider switching to less-toxic alternatives such as ***SYBR Safe™ DNA gel and SYBR Green.*** Only these two EtBr alternatives have been evaluated by the Environmental Health and Safety Department and as a result can be treated as non-hazardous materials ***requiring no special waste disposal procedures.*** If you would like to use some other alternative other than the two mentioned above contact the Environmental Health and Safety Department for an evaluation of use and disposal.

**SAFETY REFERENCES**

Additional chemical safety information, including MSDSs and other information, is available electronically as tools at [ehs.harvard.edu/programs/safe-chemical-work-practices](http://ehs.harvard.edu/programs/safe-chemical-work-practices).

**WASTE**

**Hazardous Waste**

* Ethidium Bromide **Stock Solutions** and **Powder**- or **Crystal**-contaminated materials must be managed and disposed of ***as a hazardous waste.***

**Non-Hazardous Waste:**

* + Agarose gels with trace amounts of ethidium bromide (0.3 - 0.5 μg/ml) are **not** regulated as hazardous wastes. See the waste handling approach in Appendix A on the next page.
* **Buffer Solutions** with trace amounts of Ethidium Bromide can be disposed of down the drain.
* **Pipette tips** can be disposed of in regular trash.

**EMERGENCY PROCEDURES**

(Refer to the [Emergency Response Guide](http://www.ehs.harvard.edu/programs/emergency-guidance) posted in your lab, supplemented with information here)

* All spills involving EtBr stock solutions, powder or crystals 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.
* Use gloves and wipes to clean up dropped gels or spilled buffer solution, and manage the cleanup materials using the waste handling procedure for gels in Appendix A.

**APPENDIX A: ETHIDIUM BROMIDE WASTE HANDLING DETAILS**

**Non-Hazardous Waste Streams**

|  |  |  |
| --- | --- | --- |
| **Waste Stream** | **Description** | **Waste Management Procedure** |
| **Buffer Solutions** | Typically contain very small concentrations of EtBr (< 0.01% by wt.) | May be discharged to sinks. |
| **Gels** | Typically contain trace amounts of EtBr (0.3-0.5 μg/ml) | Agarose gels with trace amounts of ethidium bromide may be disposed of ***daily***in double bag-lined containers (e.g., five-gallon pails). This waste is **not** considered a hazardous waste. However, the chemical waste vendor will pick up any vendor-supplied pails that are labeled as “Non-Hazardous Ethidium Bromide Gel Waste”. Cambridge labs that have mini-mains will have to bring the pails to the mini-mains when full. |
| **Hazardous Waste Streams**  *Contact EHS for assistance or waste removal, at 496-3797 (Cambridge) or 432-1720 (Longwood).* | | |
| **Waste Stream** | **Description** | **Waste Management Procedure** |
| **Stock Solutions**  **Crystals & Powders** | Typically contain relatively high concentrations of pure EtBr | Place empty stock solution bottles or unused stock solution bottles in double ziplock plastic bags. Place absorbents into bag if available.  Keep waste containers/bags in secondary containment bins, closed when not being added to, 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)”.  Store waste containers at hazardous waste satellite accumulation area. |
| **Contaminated Debris** | Includes EtBr-contaminated debris (e.g., gloves, mats, tips, spill clean-up waste) from spills of Stock Solution, Crystals or Powder | Collect waste in double bags.  Bags must remain closed, when not in use, and labeled as “Hazardous Waste: Ethidium Bromide Debris (Mutagen)”. When labeling, be sure to check the “Other” box under hazard type and specify “Ethidium Bromide Debris (Mutagen)”. Store waste bags at hazardous waste satellite accumulation area. |