

Standard Operating Procedure for work with Ethidium Bromide

Chemical name/class: Ethidium Bromide CAS: 1239-45-8

PI: _____ Date: _____

Building and Room number: _____ Designated Area: _____

1. **Circumstances of Use:**

Ethidium bromide is an intercalating agent commonly used as a fluorescent tag (nucleic acid stain) in molecular biology laboratories for techniques such as agarose gel electrophoresis. It is commonly abbreviated as "EtBr", which is also an abbreviation for bromoethane. When exposed to ultraviolet light, it will fluoresce with an orange color, intensifying almost 20-fold after binding to DNA.

Ethidium bromide is toxic and a mutagen. It may also be a carcinogen, or a teratogen, although this depends on the organism exposed and the circumstances of exposure.

2. **Potential Hazards:**



Ethidium bromide is classified by OSHA as highly toxic by inhalation, harmful by ingestion, and a mutagen. Avoid breathing dust, fumes, gas, vapor, mist, or spray. Toxic if inhaled. Suspected of causing genetic defects.

3. **Engineering Controls:**

All operations involving powder or mists of ethidium bromide must be done in a fume hood. Check for proper operation of the fume hood prior to use.

4. **Work Practice Controls:**

It is advisable that any laboratory that routinely works with ethidium bromide. Set up a designated area for work with ethidium bromide, and label it with the following wording: "Ethidium Bromide in use."

- Line the work area with a disposable plastic-backed absorbent pad.
- Keep containers closed as much as possible.
- If weighing dry powders and the balance cannot be located in a fume hood or BSC, tare a container then add the material to the container in a hood and seal the container before returning to the balance to weigh the powder.
- Change gloves regularly (at least every two hours) and wash hands at the time of the glove change.

When an ultraviolet light source is used in your work with ethidium bromide, added caution is required. As a general rule, avoid exposing unprotected skin and eyes to intense UV sources. If the UV light is aimed upwards, wear a UV protective face shield when you are standing near the source. For prolonged work close to UV light boxes or other intense sources, it may be useful to wrap the end of the lab coat sleeves loosely with masking tape to prevent gaps where the wrist could be exposed.

5. Personal protective equipment (PPE):**Hand Protection:**

Gloves must be worn. Use proper glove removal technique to avoid any skin contact. Nitrile gloves are recommended. For extended periods of usage, wearing two pairs of nitrile gloves is recommended.

NOTE: Consult with your preferred glove manufacturer to ensure that the gloves you plan on using are compatible with Ethidium bromide.

Eye Protection:

ANSI approved properly fitting safety glasses or chemical splash goggles.

Skin and Body Protection:

Laboratory coats must be worn and be appropriately sized for the individual and buttoned to their full length. Laboratory coat sleeves must be of sufficient length to prevent skin exposure while wearing gloves. Personnel should also wear full length pants, or equivalent, and close-toed shoes. Full length pants and close-toed shoes must be worn at all times by all individuals that are occupying the laboratory area. The area of skin between the shoe and ankle should not be exposed.

Hygiene Measures:

Wash thoroughly and immediately after handling. Remove any contaminated clothing and wash before reuse.

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6. Transportation and Storage:

Liquid: Store in the dark and the cold, preferably in a plastic container.

Solid: Store at the designated area.

Ethidium bromide powder and solutions should be in a tightly closed shatter-resistant containers during transportation and storage. Secondary containment is advised. It should be stored away from strong oxidizing agents.

7. Waste Disposal:

All solid waste composed of ethidium bromide, or solid materials that come in direct contact with ethidium bromide stock solution must be disposed of in a 5 gallon solid chemical waste container lined with a plastic bag, e.g. gloves, absorbent pads, gels, paper towels, pipette tips etc. Do not use a red biohazard bag to line the buckets. For the liquid ethidium bromide waste such as buffers, please use a 5 gallon carboy and label the container appropriately. If you would like to employ any alternative ethidium bromide disposal methods please consult with Rice University EHS before starting.

8. Exposures/Unintended contact:

- ANY accidents involving Ethidium Bromide solutions must be reported to EHS.
- In case of *skin contact*: Flush the skin with copious amounts of water for at least 15 minutes. Seek medical attention. May cause skin burns.
- In case of *eye contact*: Flush contaminated eye(s) immediately with copious amounts of water for at least 15 minutes. Seek medical attention immediately. Piranha is corrosive and can cause irreversible damage to the eyes.
- In case of *inhalation*: Assist conscious persons to an area with fresh, uncontaminated air. Seek medical attention if experiencing respiratory irritation, cough, or tightness in the chest.
- In the case of *accidental ingestion* of the Piranha solution, seek medical attention immediately! After seeking medical attention, go to <http://safety.rice.edu> and complete an Accident report form and return to EHS.

9. Spill Procedure:

Spills of ethidium bromide solutions should be absorbed and decontaminated with soap and water, or ethanol. Avoid raising dust when cleaning up solid spills by mixing with water and then absorbing the solution. All spill cleanup materials and absorbents should be bagged or placed in a sealed container with a hazardous waste label.

10. Training of personnel:

All personnel are required to complete the online General Lab Safety session. This session includes an introduction to general chemical safety.

Furthermore, training on the lab-specific procedures must be performed by the PI or knowledgeable designee for all personnel working with Piranha, and must be documented (topics covered, date, employee names and signatures). All personnel shall read and fully adhere to the laboratory-specific SOP for Piranha, and shall document that they have read it by signing and dating the SOP.

“I have read and understand this SOP. I agree to fully adhere to its requirements. By signing below, I also acknowledge that I have received hands-on training for use of Ethidium Bromide.”

Last	First	Signature	Date	Trainer Initials