Laboratory Standard Operating Procedure: Ethidium Bromide [EtBr]

I.	CONTACT INFORMATION

Procedure Title	
Procedure Author	
Date of Creation/Revision:	**April 12, 2013**
Name of Responsible Person	*(The PI, Lab Supervisor, or Autonomous Researcher)*
Location of Procedure	*(Building and room number)*
Approval Signature	*(Required if Modifications are made to this SOP)*

II.	THIS STANDARD OPERATING PROCEDURE (SOP) IS FOR A:

☐ **Specific laboratory procedure or experiment**
Examples: synthesis of chemiluminescent esters, folate functionalization of polymeric micelles, etc.

☐ **Generic laboratory procedure that covers several chemicals**
Examples: distillation, chromatography, etc.

☒ **Generic use of specific chemical or class of chemicals with similar hazards**
Examples: organic azides, mineral acids, etc.

This standard operating procedure (SOP) is intended to provide general guidance on how to safely work with Ethidium bromide. This general use SOP only addresses safety issues specific to this chemical agent. In some instances, the laboratory procedure may involve several general use SOPs. If you have questions concerning the applicability of any item listed in this procedure contact the Principal Investigator/Laboratory, Supervisor of your laboratory or the Chemical Environmental Health and Safety Coordinator [CEHSC] at (813) 842-3528.

III.	CLASS OF HAZARDOUS CHEMICALS

Ethidium Bromide (EtBr) is commonly used as a non-radioactive marker for identifying and visualizing nucleic acid bands in electrophoresis and in other methods of nucleic acid

separation.

1. Ethidium Bromide is an odorless solid that is irritating to the eyes, skin, mucous membranes, and upper respiratory tract.
2. Ethidium bromide is a potent mutagen and should be treated as a possible reproductive hazard and carcinogen.
3. Ethidium Bromide is Acutely Toxic

Consult the Safety Data Sheet [SDS] and Laboratory Chemical Safety Summary for Ethidium Bromide in Prudent Practices in the Laboratory (National Academies Press).

IV. GENERAL HAZARD CONTROL

Laboratory specific SOPs will vary according to the material used and detail of experimentation techniques. The following guidelines are to be applied as a general precaution for projects involving EtBr:

1) Use the smallest amount of chemical that is consistent with the requirements of the work to be performed.
2) Use containment devices (such as lab fume hoods or glove boxes) when:
   a) Volatilizing these substances,
   b) Manipulating substances that may generate aerosols, and
   c) Performing laboratory procedures that may result in uncontrolled release of the substance.
3) Use ventilated containment to weigh out solid chemicals.
4) Alternatively, the tare method can be used to prevent inhalation of the chemical.
   a) While working in a laboratory hood, the chemical is added to a pre-weighed container.
   b) The container is then sealed and can be re-weighed outside of the hood.
   c) If chemical needs to be added or removed, this manipulation is carried out in the hood. In this manner, all open chemical handling is conducted in the laboratory hood.

V. ENGINEERING & VENTILATION CONTROLS

Stock solutions of EtBr should be prepared in a properly functioning lab fume hood. If aerosols may be produced (dust or liquid mist), Ethidium bromide must be handled in a chemical fume

hood, exhausted biological safety cabinet with negative pressure ductwork, or other exhausted enclosure. Aerosols may be produced during any open handling of dry powder, and during open or pressurized manipulations of solutions. If the process does not permit the handing of such materials in a fume hood, contact the CEHSC at (813) 842-3528 for reviewing the adequacy of room ventilation standards..

Follow Safe Fume Hood Practices:

1. Ensure the fume hood's certification date is within a one-year period. Verify sufficient inward airflow before using a hood by checking the hood's airflow indicator. Report any problems to PI/Lab Supervisor and the CEHSC.
2. Maintain hood sash at or below the maximum height indicated by an arrow on the side of the fume hood.
3. Close the hood sash when not working in the hood.
4. Avoid rapid movements at the face of the hood to avoid creating competing air currents that reduce the ability of the hood to contain air contaminants.
5. Equipment used in hoods should be placed securely on blocks to allow air to flow under and around the equipment.
6. Keep chemical sources and equipment at least six inches away from the face or rear of the hood.
7. Minimize equipment and chemical storage placed in the hood to avoid dead air spaces or eddies and to prevent blocking back baffles.

VI. PERSONAL PROTECTIVE EQUIPMENT

☒ Lab Coat ☒ Long pants ☒Close-toed shoes ☒Safety glasses

☒ Nitrile Gloves

The above listed personal protective equipment should be worn when handling Ethidium bromide. Additional protection may be required based upon additional experimental

chemical agent(s) in use.

At minimum, nitrile gloves, safety glasses, lab coat, long pants, and closed toed shoes are to be worn when work tasks involve EtBr. Additionally:

1. When handling these chemicals or contacting potentially contaminated surfaces, protective gloves are to be worn. For proper selection of glove material, review **chemical SDS** and **glove selection guidance**.
2. Double nitrile gloves are suggested:
    a. Change outer gloves frequently to minimize cross-contamination.
    b. Immediately replace with new gloves when splash occurs.
3. Goggles (not safety glasses) are appropriate for processes where a splash or spray potential exists.
4. Impervious chemical resistant apron/smock/lab coat (PE or PVC) should be worn when handling pure EtBr and concentrated stock solutions
    a. Avoid using the traditional cotton-polyester white lab coat, which readily collects/absorbs compounds.
5. Additional protective clothing (i.e., face shield, apron, and oversleeves) is appropriate for chemicals that are toxic through skin contact or dermal absorption.
6. When using a UV light to visualize EtBr contamination, wear UV-blocking eyewear or work behind a UV shielding glass. (Most standard safety glasses will block UV, but employees should check the approval of their safety glasses.)

VII.	SPECIAL HANDLING PROCEDURES AND STORAGE REQUIREMENTS

Ensure secondary containment and segregation of incompatible chemicals per guidance within the **UT Chemical Hygiene Plan**. Also, follow any substance-specific storage guidance provided in SDS documentation.

Safe Handling	**1. Line the work area with a disposable plastic-backed absorbent pad.** **2. Keep containers closed as much as possible.** **3. If weighing dry powders and the balance cannot be located in a fume hood or biological safety cabinet, 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.** **4. Change outer glove layer regularly (at least every two hours) and wash hands at the time of the glove change.**

	5. Natural rubber latex gloves do <u>not</u> provide a suitable barrier to penetration by EtBr. **6. 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.** **7. 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.**
Safe Storage	**1. EtBr powder and solutions should be in tightly closed-shatter-resistant containers during transportation and storage. Secondary containment is advised.** **2. EtBr should be stored away from strong oxidizing agents in a cool, dry place.** **EtBr should be stored in a cool, dry place and the container must be kept undamaged and tightly closed.**

VIII. DESIGNATED AREA

A designated area shall be established when using EtBr, where limited access, special procedures, knowledge, and work skills are required. A designated area can be the entire laboratory, a specific laboratory workbench, or a laboratory hood. Designated areas must be clearly marked with signs that identify the chemical hazard and include an appropriate warning; *for example*: **WARNING! ETHIDIUM BROMIDE IN USE. MUTAGEN AND IRRITANT**

1. Upon leaving the designated area, remove any personal protective equipment worn and wash hands, forearms, face, and neck.
2. After each use (or day), wipe down the immediate work area and equipment to prevent accumulation of chemical residue.
3. At the end of each project, thoroughly decontaminate the designated area before resuming normal laboratory work in the area.

IX. EMERGENCY SPILL AND ACCIDENT PROCEDURES

Health-Threatening Emergencies	1. **Call 911**

Examples: Fire, explosion, health-threatening hazardous material spill or other Immediate Danger	2. Alert people in the vicinity to evacuate 3. Activate the local alarm systems 4. Remain nearby to provide arriving emergency responder information about chemicals 5. Once personal safety is established, call the CEHSC at (813) 842-3528
Personnel Injury or Exposure	1. Remove the injured/exposed individual from the area if it is safe to do so because of the medical condition of the victim or the potential hazard to rescuers. 2. **Call 911** 3. Administer first aid as appropriate. 4. Flush contamination from eyes/skin using the nearest emergency eyewash/shower for a minimum of 15 minutes. 5. Remove any contaminated clothing to prevent contaminants from continuing to absorb onto skin. 6. Give medical responders copies of SDSs for all chemicals the victim was exposed to. 7. Report the exposure to the CESHC
Non-Health Threatening Emergencies	Call Security at 813-257-7777 or xtn. 7777 to report incident
Small Spill Clean-Up	**Note: Only minor spills or releases can be cleaned up by knowledgeable personnel using readily available equipment:** 1. Notify personnel in the area and restrict access. Eliminate all sources of ignition. 2. Review the SDS for the spilled material, or use your knowledge of the hazards of the material to determine the appropriate level of protection. 3. For spills of liquid suspensions, absorb the spilled material with paper towels or other absorbent, and then decontaminate the area. 4. For small spills of powdered or

	granular EtBr, carefully wipe up with wet paper towels, and then follow one of the decontamination surface area methods. 5. Do not use bleach solutions to clean up EtBr contamination. It is not effective and can be explosive under certain conditions. 6. If greater than 30 ml, or if it will take longer than 15 minutes for you to clean-up, immediately call Security (813) 257-7777 or xtn. 7777 to report the spill, and notify your supervisor. 7. Collect all waste absorbent and PPE into a bag and seal. 8. Submit waste pickup request to the CEHSC.
Incident Reporting	1. Report all occupational injuries or illness to laboratory supervisor as soon as practical. 2. Laboratory personnel are encouraged to report "near misses" as they are considered a precursor to actual incidents. 3. Laboratory supervisor is to conduct (or coordinate) an investigation of all incidents and "near misses." The goal of the investigation is to identify and address any deficiencies that may have contributed to the incident.
Medical Consultation	Laboratory personnel who work with hazardous chemicals are to be provided the opportunity to receive medical attention/consultation when: 1. A spill, leak, explosion or other occurrence results in a hazardous exposure (potential overexposure); or 2. Symptoms or signs of exposure to a hazardous chemical develop. 3. Contact your CEHSC for more information or directions to medical services.

X.	WASTE DISPOSAL

Ethidium bromide is commonly used in molecular biology laboratories. While not regulated as a "hazardous waste", the mutagenic properties of this substance may present a hazard if improperly disposed of down the drain or placed into the trash. Based on these considerations, the following disposal procedures are recommended:

1. Dilute Aqueous Solutions
    a. Solutions containing less than 10 µg/ml Ethidium bromide by weight may be discharged to sinks, while flushed with copious amounts of water.
    b. Solutions containing more than 10 µg/ml by wt. can be:
        i. Filtered through commercially available carbon filter cartridges to remove Ethidium bromide.
        ii. Spent cartridges or bags must be collected in waste containers labeled with the words "Waste Ethidium Bromide (Mutagen)" and submitted to CEHSC for proper disposal, or
        iii. Solutions that are not filtered in the laboratory should be collected in containers that are labeled with the words "Waste Ethidium Bromide (Mutagen)" and submitted to CEHSC for proper disposal.
2. Ethidium Bromide Gels –
    a. Spent Ethidium bromide gels that are colorless may be:
        i. collected and double-bags and placed into a waste container labeled with the words "Waste Ethidium Bromide (Mutagen)" and submitted to CEHSC for proper disposal, or
        ii. Placed into autoclave bags, autoclaved, and disposed of as solid waste.
    b. All other gels should be collected in double-bags and placed into a waste container labeled with the words "Waste Ethidium Bromide (Mutagen)" and submitted to the CESHC for proper disposal.
3. Unused Stock Solutions, Crystals, and Powders –
    a. Collect in waste containers labeled with the words "Waste Ethidium Bromide (Mutagen)" and submit to CEHSC for proper disposal.
4. Contaminated Debris – Contaminated gloves, mats, tips, or spill clean-up waste should be collected in 4mm plastic bags, double-packed if necessary, labeled as "Waste Ethidium Bromide (Mutagen)", and submitted to CEHSC for proper disposal.

Please call the CEHSC at (813) 842-3528 to describe the quantities of waste you anticipate generating and appropriate waste disposal procedures. Include any special handling or storage requirements for your waste.

XI.	DECONTAMINATION PROCEDURES
Personnel	Immediately after working with sensitizers, remove gloves, wash hands and arms with soap and water.
Area	1. Check the work area for contamination using a UV light (EtBr will fluoresce a reddish-brown). If decontamination

	is needed, try the methods below after wiping up excess liquid with paper towels. 2. Wipe the contaminated area or equipment with fresh towels and a soap/water solution multiple times. You can also wipe with towels soaked in ethanol. Check for any remaining contamination using UV light. 3. Take fresh paper towels soaked in ethanol and place them over the contaminated surface. Sprinkle activated charcoal on the ethanol-saturated towel in contact with contaminated surface. Wipe up ethanol/charcoal mixture with additional towels and place all clean-up materials into a plastic bag. Check for any remaining contamination with UV light and repeat if needed. 4. Use a solution of 4.2 grams of sodium nitrite ($NaNO_2$), 20 milliliters of 50% hypophosphorous acid solution (H_3PO_2), and 300 milliliters of water to decontaminate. Check the area again with the UV light until all EtBr has been removed, then rinse with water. It should be noted that hypophosphorous acid is a DEA listed chemical and may require additional authorization for purchase.
Equipment	Decontaminate vacuum pumps or other contaminated equipment (glassware) before removing them from the designated area.
XII.	**TRAINING REQUIREMENTS**

General Training *(check all that apply):*

- ☑ General Safety & Emergency Preparedness – Annual Orientation Training
- ☑ Review of SDS for other chemicals involved in process/experiment
- ☑ Review of this SOP
- ☐ Other: _____

The University may require additional safety training depending on the hazardous materials and laboratory-specific processes – consult the PI for more information.