

Biology and Wildlife
STANDARD OPERATING PROCEDURE
Ethidium Bromide



This SOP addresses only ethidium bromide. Users must also follow the electrophoresis SOP and any other SOPs relevant to their particular procedure (e.g. UV light box use, etc.).

IMPORTANT NOTE: Ethidium bromide use has been discontinued in Biology and Wildlife laboratories. Alternatives such as GelRed and SybrGreen are safe and effective alternatives, making the risks associated with ethidium bromide unwarranted. If you believe that your procedure requires ethidium bromide to visualize your results, you must get permission in advance from the Laboratory Supervisor to ensure that appropriate safety and decontamination procedures are in place.

Location(s): Murie 204, 206, 306

Chemical(s): Ethidium Bromide, CAS # 1239-45-8

Specific Hazards:

- GHS Classification in accordance with [29 CFR 1910.1200](#) (OSHA HCS):
 - Acute toxicity, oral (category 4), H302
 - Acute toxicity, inhalation (category 2), H330
 - Germ cell mutagenicity (category 2), H341
 - Suspected of causing genetic defects.
- Danger. Fatal if swallowed. Do not breathe dust / fume / gas / mist / vapors / spray. Wash skin thoroughly after handling and removing gloves. Use only in a well ventilated area.
- Do not handle it until all safety precautions have been read and understood.

Contact Information:

Laboratory Supervisor: Patrick Knavel pdknavel@alaska.edu 474-5622

Laboratory Technician: Rosa Villarreal rvillarreal@alaska.edu 474-7205

1. Purchasing:

If ethidium bromide is needed, the Laboratory Supervisor and Laboratory Technician should be contacted well in advance of the date of the lab. Both must be contacted so that the purchase and the relevant safety concerns can be addressed.

2. Storage:

Locked in 209 or 215 Murie chemical cabinets for long-term storage.

Locked in the cabinet in 306, 204, or 206 when needed for a specific lab exercise.

3. Authorized personnel:

- All authorized personnel must have completed all required employee and laboratory safety training.
- The Instructor is authorized to train their TAs on the proper preparation, handling, storage and disposal of this material. The instructor may delegate training to the B&W Laboratory Supervisor by making arrangements at least two (2) weeks in advance.
- TAs, once trained, are authorized to train and supervise their students.
- Students must be trained in the use of this material in accordance with this SOP before they run gels.

4. Training requirements:

The user must demonstrate competency and familiarity regarding the safe handling and use of these materials prior to using them. Training shall include the following:

- Review of this SOP
- In-person review of procedures.

5. Use location:

- Murie B&W teaching labs, rooms 204, 206, 306
- On tables or lab benches isolated from sinks.
- This material shall NOT be used near a sink. In the event of a leak or spill, this material must be contained and may not enter the drain.

6. Personal protective equipment (PPE):

- All personnel are required to wear the following personal protective equipment (PPE) whenever conducting this procedure:
 - Nitrile gloves, thickness of 0.11mm
 - Safety goggles
 - Lab coat, long sleeved
- PPE must be inspected prior to use and replaced if damaged.
- PPE must be removed as appropriate to avoid contaminating surfaces and items in the lab or outside of the lab that should not be contaminated. In particular, PPE must be removed before leaving the lab, before handling personal items such as cell phones or laptops, and before moving on to other procedures in the lab. If a subsequent lab procedure also requires gloves, ethidium bromide-contaminated gloves must be removed and disposed of appropriately, and fresh gloves must be donned.

7. Spill equipment:

- Absorbent bench paper shall be used to cover the benchtops in areas where this material will be used. Bench paper must be secured to the table (masking tape is acceptable), absorbent side up and plastic barrier side down. Bench paper must be placed in areas where ethidium bromide containing solutions and/or gels will be manipulated and under gel rigs that will contain ethidium bromide.
- PPE as specified in section 6 of this document.
- Inert absorbent material. Paper towels are suitable.
- Waste containers to keep contaminated material separate from trash.

In the event of a spill, follow the directions in section 12, below.

8. Procedure:

Materials needed:

- ethidium bromide
- agarose and gel casting trays or precast agarose gels
- pipet and pipet tip (use an ethidium bromide designated pipet as it is difficult to fully decontaminate pipets)
- absorbent bench paper
- masking tape
- waste container for collecting liquid waste
- waste container for collecting solid waste (including gels)

Procedure Notes:

PPE must be used appropriately throughout the procedures.

Procedure Steps:

1. Don appropriate PPE. Mark off and set up a work area.
2. The TA will add ethidium bromide to the gel and/or buffer following the laboratory protocol provided by the instructor. Ethidium bromide should be handled in the fume hood; B&W uses concentrated solutions to prepare gels and running buffers, which can be used on an appropriately protected laboratory bench. Absorbent bench paper should be placed in the fume hood to absorb any spills.
3. During the lab, work will be done in a designated ethidium bromide workspace on a benchtop or counter in the lab that is protected with absorbent bench paper. This workspace shall be clearly labeled "Danger: Ethidium Bromide Area. Appropriate PPE and training required." All contaminated equipment shall remain in this area and on the absorbent bench paper. All wastes produced shall be collected appropriately for disposal as hazardous waste (see below).
4. Ethidium bromide containing gels shall be stored prior to use in a sealed, leak proof container. This container shall be clearly labeled with the contents, hazard, course, instructor, and date of preparation. Secondary containment shall be used to prevent contamination of surfaces.
5. The TA or instructor shall set up the electrophoresis chamber by placing the gel in the rig and adding a buffer. This should not be delegated to students.
6. When loading samples containing ethidium bromide, students shall take turns using a pipet designated for use with ethidium bromide. They should NOT use multiple pipets as this contaminates many pipets and thorough decontamination of pipets is difficult. Pipet tips must be collected in a designated, labeled waste container at the ethidium bromide work space.
7. Follow the SOP for electrophoresis and any other relevant SOPs for any other hazardous materials used.
8. After electrophoresis is complete and the power turned off and units unplugged, gels will need to be visualized. PPE must be worn when transferring gels out of the buffer. Gels should be contained in trays or dishes that prevent ethidium bromide contamination of surrounding surfaces.
9. Used buffers contaminated with ethidium bromide shall be transferred to a leak proof waste container that can be tightly sealed. The waste container shall be in secondary containment that is large enough to hold the entire volume of buffer collected. Transfer from the chamber to the waste container shall be done over a bin or container that is sufficient to catch any drips or spills. Transfer of contaminated buffers shall NEVER take place in, over or near a sink, as it cannot be allowed to enter the drain.

9. Waste disposal and clean up:

- All contaminated equipment (including glassware) shall be kept in the designated work space. It shall NOT be washed or cleaned by the students, TAs or instructors.
- Collect used buffers in an appropriate container. Containers shall be obtained in advance from the B&W Laboratory Technician.
- Containers must be kept securely capped and in secondary containment that is sufficient to hold more than the volume of the primary waste container.
- Label the waste container with “Waste (*specify type of buffer*) contaminated with ethidium bromide,” the course, the instructor name, and the date that collection of waste began.
- Used, contaminated gels, pipet tips, gloves, etc. shall be collected in an appropriate waste container labeled with the gel type (e.g. 1% agarose), Ethidium Bromide Contaminated, the course, the instructor name, and the date collected.
- The Laboratory Technician shall be notified in advance of the expected completion date of the lab. When waste is ready for pick-up, the Laboratory Technician shall be contacted by the instructor or TA running the lab to confirm that the waste is ready for pick-up, and equipment is ready for decontamination.

10. Decontamination:

- Neither students nor TAs nor instructors will carry out decontamination.
- Decontamination of equipment shall be carried out by the Laboratory Technician and Laboratory Supervisor. They shall follow the approved procedure for decontamination that is used by IAB.
- Decontamination of used buffers shall be carried out by the Laboratory Technician and Laboratory Supervisor. They shall follow the approved procedure for decontamination that is used by IAB.
- If equipment or a portion of the room outside of the ethidium bromide work area becomes contaminated, it is the responsibility of the TA or instructor teaching the lab to clearly mark off the area or equipment, label it “Danger: Ethidium Bromide contamination – DO NOT TOUCH” and notify the Laboratory Technician, Laboratory Supervisor, and others who will be using the room immediately. The Laboratory Technician and Laboratory Supervisor will carry out or arrange for decontamination.

11. Exposures: Emergency procedures to be followed (from SDS):

The most important known symptoms and effects are as stated in the “Specific Hazards” statement at the beginning of this document.

General advice

Consult a physician. Show the safety data sheet to the doctor in attendance. Move out of the dangerous area.

Eye contact with ethidium bromide solutions

Flush eyes with water as a precaution. Consult a physician.

Skin contact with ethidium bromide solutions

Wash off with soap and plenty of water. Take the victim immediately to hospital; call 911 for transport if necessary. Consult a physician. Provide the physician with a copy of the Safety Data Sheet.

Ingestion of ethidium bromide solutions

Never give anything by mouth to an unconscious person. Rinse mouth with water. Immediately consult a physician.

Inhalation

If breathed in, move the person into fresh air. Immediately consult a physician. If not breathing, give artificial respiration and call 911.

12. Spills:

- If a spill occurs, personal safety should come first.
- Alert everyone in the area where the spill occurred. Students should be directed to move out of the way and allow the TA to clean up the spill.
 - If the spill occurred on the absorbent bench paper and the paper was sufficient to absorb the spill, the equipment on the wet paper should be moved to clean bench paper. The wet bench paper can be carefully collected to prevent the spill from contacting any surfaces. The contaminated paper shall be disposed of as solid hazardous waste.
 - If the spill occurred outside the designated area, paper towels should be placed on the spilled liquid to absorb it. These paper towels must be collected and disposed of as hazardous waste. The area of the spill should be taped off and warning signs clearly posted to indicate the contaminated area and identify the hazard and contaminant. The Laboratory Technician and Laboratory Supervisor must be notified immediately so that decontamination can take place.
- If the spill occurs near an electrophoresis unit, shut off and unplug the power unit if it is safe to do so. Shut off and unplug any other electrical equipment in the area that could pose a shock hazard.
- Do not allow ethidium bromide to enter drains. If necessary, place absorbent material between the spill and any drain it could enter.
- Any surfaces that come into contact with ethidium bromide solutions are contaminated until decontaminated, and must be clearly marked off and labeled as contaminated. Warning signs must be posted to alert those who could come into contact with the contaminated surfaces of the contaminant and hazards it poses. The Laboratory Technician and Laboratory Supervisor must be immediately notified of any contamination.

13. Phone numbers:

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| Biology and Wildlife Laboratory Supervisor | 474-5622 |
| Biology and Wildlife Laboratory Technician | 474-7205 |
| EHSRM Hazardous Materials (if Lab Supervisor not available; assistance with spills) | 474-5617 |
| EHSRM Industrial Hygiene (if HazMat not available; assistance with exposures) | 474-6771 |
| EHSRM office (if Hazardous Materials or Industrial Hygiene not available) | 474-5413 |
| University of Alaska Fairbanks Emergency Response (serious accidents, fire) | 911 |

14. Other important information:

This material must not enter the standard solid or liquid waste streams (i.e. regular trash or sink drains). All contaminated materials must be collected and disposed of as hazardous waste.

