



## Standard Operating Procedure

### Ethidium Bromide

Researchers should not use Ethidium Bromide until they have read and fully understood these safe operating procedures. However, reading these procedures does not substitute for hands-on training. New users of Ethidium Bromide must work under the close supervision of an experienced user.

BEFORE working with Ethidium Bromide, read the relevant Material Safety Data Sheets (MSDS)/ Safety Data Sheets (SDS) and understand the hazards. The safety sheet must be reviewed before using an unfamiliar chemical and periodically as a reminder.

#### 1. This standard operating procedure (SOP) is for a

Specific laboratory procedure or experiment

Examples: synthesis of chemiluminescent esters, folate functionalization of polymeric micelles

Generic laboratory procedure that covers several chemicals

Examples: distillation, chromatography

Generic use of specific chemical or class of chemicals with similar hazards

Examples: organic azides, mineral acids

#### 2. Chemical Description

Ethidium Bromide is an organic compound with the formula  $C_{21}H_{20}BrN_3$ . Ethidium bromide, (Dromilac, homidium bromide), CAS # 1239-45-8, is a material that fluoresces a red-orange color under ultraviolet light, with increased fluorescence when the material is bound to double-stranded DNA. Ethidium bromide is typically purchased in powder or solution form and is soluble in water. The crystal or powder form is odorless and appears dark red in color.

The powder form is considered an irritant to the upper respiratory track, eyes and skin. Ethidium bromide is strongly mutagenic, causing living cell mutations. This material is considered a possible carcinogen or teratogen. commonly used in molecular biology laboratories for visualizing nucleic acids using electrophoresis and other gel-based nucleic acid separation methods. It can be readily absorbed through the skin and mucus membranes. Avoid direct skin contact!



3.	<b>Risk assessment</b>
	Ethidium Bromide is a strong health hazard and has strict decontamination, personal protective equipment and disposal protocols. It is recommended to substitute a safer alternative such as SYBR Safe™ DNA gel and SYBR Green.
4.	<b>Symptoms of Ethidium Bromide Exposure</b>
	<p>Ethidium Bromide is extremely corrosive. It is harmful if inhaled, ingested, or absorbed through the skin.</p> <ol style="list-style-type: none"> <li>1. <u>Eye Contact</u>: If applied to the eyes, this material causes severe eye damage.</li> <li>2. <u>Inhalation</u>:       <ol style="list-style-type: none"> <li>a. Inhalation of dusts, generated by the material, during the course of normal handling, may produce severely toxic effects; these may be fatal.</li> <li>b. The material is not thought to produce respiratory irritation (as classified by EC Directives using animal models). Nevertheless, inhalation of dusts, or fumes, especially for prolonged periods, may produce respiratory discomfort and occasionally, distress.</li> <li>c. Persons with impaired respiratory function, airway diseases and conditions such as emphysema or chronic bronchitis, may incur further disability if excessive concentrations of particulate are inhaled.</li> <li>d. If prior damage to the circulatory or nervous systems has occurred or if kidney damage has been sustained, proper screenings should be conducted on individuals who may be exposed to further risk if handling and use of the material result in excessive exposures.</li> <li>e. Heating of Ethidium Bromide compounds increases inhalation exposures and equipment contamination.</li> </ol> </li> <li>3. <u>Ingestion</u>:       <ol style="list-style-type: none"> <li>a. Accidental ingestion of the material may be harmful; animal experiments indicate that ingestion of less than 150 gram may be fatal or may produce serious damage to the health of the individual.</li> <li>b. Bromide poisoning causes intense vomiting so the dose is often removed. Effects include drowsiness, irritability, incoordination, vertigo, confusion, mania, hallucinations and coma. Other effects include skin rash, nervous system symptoms, sensory disturbances and increased spinal fluid pressure. They have been used as sedatives and depress the central nervous system. Toxicity is increased if dietary chloride is reduced. Repeated ingestion can cause a syndrome with acne, confusion, irritability, tremor, memory loss, weight loss, headache, slurred speech, delusions, stupor, psychosis and coma.</li> <li>c. Concentrated solutions of many cationics may cause corrosive damage to mucous membranes and the oesophagus. Nausea and vomiting (sometimes bloody) may follow ingestion. Serious exposures may produce an immediate burning sensation of the</li> </ol> </li> </ol>

mouth, throat and abdomen with profuse salivation, ulceration of mucous membranes, signs of circulatory shock (hypotension, laboured breathing, and cyanosis) and a feeling of apprehension, restlessness, confusion and weakness. Weak convulsive movements may precede central nervous system depression. Erosion, ulceration, and petechial haemorrhage may occur through the small intestine with glottic, brain and pulmonary oedema. Death may result from asphyxiation due to paralysis of the muscles of respiration or cardiovascular collapse. Fatal poisoning may arise even when the only pathological signs are visceral congestion, swallowing, mild pulmonary oedema or varying signs of gastrointestinal irritation. Individuals who survive a period of severe hypertension may develop kidney failure. Cloudy swelling, patchy necrosis and fatty infiltration in such visceral organs as the heart, liver and kidneys shows at death.

4. Skin Contact:

- a. This material can cause inflammation of the skin on contact in some persons.
- b. The material may accentuate any pre-existing dermatitis condition.
- c. Skin contact with the material may damage the health of the individual; systemic effects may result following absorption.
- d. Open cuts, abraded or irritated skin should not be exposed to this material.
- e. Entry into the blood-stream, thru, for example, cuts, abrasions or lesions, may produce systemic injury with harmful effects. Examine the skin prior to the use of the material and ensure that any external damage is suitably protected.

5. **Safety equipment**

5.a. Engineering Controls: Ethidium Bromide should be used in a fume hood when working with stock solutions and making formulations and dilutions. Even when working with small amounts of dilute Ethidium Bromide, the best practice is to work in a fume hood because of the splash protection the sash provides and the ability of the hood to contain emissions especially in the event of a spill.

5.b. Personal Protection Equipment: **All PPE should be inspected for wear, cracks or tears**

- a. Eye/Face Protection: Chemical splash goggles that meet the ANSI Z.87.1 1989 standard must be worn whenever handling Ethidium Bromide.
  - i. Ordinary prescription glasses will NOT provide adequate protection unless they also meet this standard.
  - ii. Fume hood sash shall be properly positioned to provide splash, spray and mist protection.
  - iii. A face shield (in addition to goggles) may also be necessary. Consider that small facial burns caused by splatter may not be life threatening but can result in permanent disfiguration.
- b. Skin Protection: Lab coat, long sleeves, closed toe shoes, long pants at a minimum.
  - i. It is recommended that disposable lab coats that remain in the designated work area be worn when working with Ethidium Bromide.

	<ul style="list-style-type: none"> <li>ii. Temporary lab coats should be disposed of as Ethidium Bromide waste, especially for potential or actual splash or exposure occurs.</li> <li>iii. Double layers of nitrile gloves required</li> <li>iv. Change outer gloves frequently to minimize cross-contamination.</li> <li>v. Immediately replace with new gloves when splash occurs.</li> <li>vi. Natural rubber latex gloves do <u>not</u> provide a suitable barrier to penetration by Ethidium Bromide.</li> </ul> <p>c. Special NOTE: Short wave ultraviolet radiation will harm your eyes and skin. When using ultraviolet light to visualize Ethidium Bromide, the user <u>should wear UV-blocking eyewear</u>, full-face shield, long-sleeve protective clothing and gloves for protection.</p>
5.c.	<b>Designated area</b>
	<ol style="list-style-type: none"> <li>1. <u>Emergency Showers and Eyewashes</u>: Any laboratory using Ethidium Bromide (or any corrosive/caustic chemical) must have an emergency eyewash station accessible within 10 seconds and located in the same room the hazard is being used. Emergency showers must be accessible within 10 seconds and can be located within the room or in the hall way.</li> <li>2. <u>Administrative Controls</u>: Never work alone when using Ethidium Bromide. Procedures requiring the use of Ethidium Bromide should have written safety SOPs associated with them.</li> <li>3. <u>Fire Extinguisher</u> <ul style="list-style-type: none"> <li>a. A Class ABC fire extinguisher must be available within 10 seconds travel time from where Ethidium Bromide chemicals are used.</li> <li>b. If a Class ABC sand may be used for small fires</li> </ul> </li> </ol> <p>DO NOT attempt to extinguish large fires or if you are not comfortable to extinguish fires</p>
6.	<b>Transport, and storage, receiving requirements</b>
	<ol style="list-style-type: none"> <li>1. Ethidium Bromide must be stored separately from flammables and reducing and oxidizing agents in an approved chemical safety cabinet. Use secondary container to store Ethidium Bromide.</li> <li>2. Store in a cool, dry, well-ventilated area away from incompatible substances.</li> <li>3. Avoid dust formation and control ignition sources</li> <li>4. Store containers on shelves below eye level</li> </ol>
7.	<b>Special Handling and Decontamination Procedures</b>
	<ul style="list-style-type: none"> <li>a. Special Handling</li> <li>b. Locations where ethidium bromide is used or stored must be identified as "Designated Areas" and demarcated with either printed Designated Area or yellow tape with "DESIGNATED AREA" written upon it.</li> </ul>

- c. Procedures requiring the use of ethidium bromide powder or having the potential to generate aerosols must be performed in a fume hood.
- d. During normal use, small spills may occur and residues may build up on equipment and other laboratory surfaces.
- e. A solution of soap and water is recommended for cleaning small spills and removing residues on equipment and laboratory surfaces.
- f. Surfaces should be covered with disposable covering to reduce contamination
- g. Due to the potential for equipment contamination, **ethidium bromide-containing agarose gel should not be heated in a microwave.**

## 2. Decontamination Procedures

### a. Decontamination Solution:

Prepare the decontamination solution just prior to use. The solution is acidic, so wear PPE when preparing/using solution.

- i. The solution consists of:
- ii. 4.2 g of sodium nitrite ( $\text{NaNO}_2$ , CAS # 7362-00-0)
- iii. 20 ml of hypophosphorous acid (50%) ( $\text{H}_3\text{PO}_2$ , CAS # 6303-21-5)
- iv. 300 ml of water
- v. Wash the area with a paper towel soaked in decontamination solution. Then rinse the area 5 times with paper towels soaked with tap water, using a fresh towel each time.
- vi. Soak all the towels in decontamination solution for one hour. Then remove them, gently wring out excess solution, place in a sealed bag or container.
- vii. If the acid may damage the contaminated surface, use a few additional rinses.

### b. Another Solution:

- i. Isopropyl Alcohol then
- ii. Radiac Wash then
- iii. Soap and Water
- iv. Using a UV light, check the area to ensure that all the EB has been removed.
- v. Contact EHS for pick up.

8.

## First Aid

1. Eye:
  - a. **Rapid and immediate decontamination is critical.**
  - b. Flush with copious amounts of water for at least 15 minutes, lifting eyelids occasionally.
  - c. Remove contact lenses if easily removable without additional trauma to the eye. Do not interrupt flushing.
  - d. Get medical attention immediately.
  - e. Tell the lab PI and Teri Anderson (505-362-7833)
  - f. Provide the SOP and SDS to emergency responders
2. Inhalation
  - a. Responder should immediately help victim to fresh air if it is safe to do so
  - b. Call 911 and tell them you have an Ethidium Bromide exposure
  - c. Tell the lab PI and Teri Anderson (505-362-7833)
  - d. Provide the SOP and SDS to emergency responders
3. Ingestion:
  - a. Do not induce vomiting.
  - b. Call 911 and tell them you have an Ethidium Bromide exposure
  - c. Tell the lab PI and Teri Anderson (505-362-7833)
  - d. Provide the SOP and SDS to emergency responders
4. Skin Contact:
  - a. If skin contact occurs, and/or skin or clothing are on fire, immediately drench in the safety shower with copious amounts of water for no less than 15 minutes to remove any remaining contaminants.
  - b. If possible to do so without further injury, remove any remaining jewelry or clothing.
  - c. Call 911 and tell them you have been exposed to Ethidium Bromide and give your exact location
  - d. Tell the lab PI and Teri Anderson (505-362-7833)
  - e. Provide the SOP and SDS to emergency responders
5. Administer first aid as appropriate.
  - a. Alert people in the vicinity
  - b. Remain nearby to advise emergency responders.
  - c. Contact EHS, UNM Police, PI, and Chemical Safety Coordinator.

9.	<b>Emergency procedures</b>
	<ol style="list-style-type: none"> <li>1. <b>Small liquid spills (&lt;50 ml )</b> <ol style="list-style-type: none"> <li>a. If you do not feel comfortable cleaning up the spill, call Teri or EHS for help (<u>never put yourself at risk!</u>)</li> <li>b. May be absorbed with sand, vermiculite or other noncombustible absorbent material.</li> <li>c. Pick up (use plastic scoops; do not use combustible materials such as corn whisks or brooms)</li> <li>d. Spills of ethidium bromide solutions should be cleaned using absorbent pads followed by surface decontamination using soap and water.</li> <li>e. Place in a sealed container for proper disposal as hazardous waste. Do not dump down the drain or into the trash.</li> </ol> </li> <li>2. If the spilled material is heated or is greater than 50 ml           <ol style="list-style-type: none"> <li>a. Remove ignition sources</li> <li>b. Evacuate the laboratory</li> <li>c. Close the doors</li> <li>d. Call Teri (362-7833) or Bobby (604-6102) or EHS (277-2753 or [afterhours] 951-0194) or UNM Police at 277-2241 or dial 911.</li> </ol> </li> <li>3. <b>Dry spills</b> <ol style="list-style-type: none"> <li>a. If you do not feel comfortable cleaning up the spill, call Teri for help (never put yourself at risk!)</li> <li>b. Clean up spills in a manner that does not disperse dust into the air Spilled dry material should first be covered with moist absorbent pads to avoid generation of dust.</li> <li>c. Spills of ethidium bromide solutions should be cleaned using absorbent pads followed by surface decontamination using soap and water.</li> <li>d. Pick up spill (use non-sparking equipment; do not use combustible materials such as corn whisks or brooms)</li> <li>e. Place in a sealed container for proper disposal as hazardous waste. Do not dump down the drain or into a waste basket.</li> </ol> </li> </ol>
10.	<b>Waste disposal</b> <i>Identify amounts of waste anticipated and appropriate disposal procedures. Segregate waste by hazard class (for example, flammable, corrosive) and state (solid, liquid), label appropriately, and place in the laboratory's hazardous waste cabinet.</i>
	<ol style="list-style-type: none"> <li>1. <b><u>Disposal of Ethidium Bromide solid contaminated material</u></b> <ol style="list-style-type: none"> <li>a. Pipet tips, gloves and other contaminated debris should be collected as hazardous waste.</li> </ol> </li> </ol>

- b. Bags are ok for dry solids, as long as the bags are sealed closed and labeled properly and there are no free-flowing liquids.
- c. Sharps (needles) must go in puncture-resistant containers.
- d. Do not place dry solids contaminated with chemicals in red or orange biohaz bags.

**2. Disposal of Ethidium Bromide and waste containing Ethidium Bromide**

- a. Ethidium Bromide compounds in manufacture’s label may be disposed of as hazardous waste
  - i. Containers must be in good condition or bagged to prevent spillage
  - ii. Lids must fit and be closed when not in use or for pick up
- b. If Ethidium Bromide is part of a mixture may be disposed of as hazardous waste
  - i. Containers must be compatible with the mixture of waste
  - ii. Containers must be in good condition
  - iii. Containers must remain closed when not in use and for pick up
  - iv. All secondary containers for waste must be labeled with the:
    - 1. Hazardous Waste Label
    - 2. Listing of Contents of the waste
    - 3. Hazards of the mixture (EHS labels have boxes to check for these for ease of use)
    - 4. Large 5-gallon metal cans should not be filled more than 75% of capacity to allow for expansion

3. Fill out the Waste Pickup Request located at <https://ehs.unm.edu/waste-management/index.html>  
 Waste label templates are located at <https://ehs.unm.edu/waste-management/index.html>

**11. Training requirements**

*List the general and laboratory-specific training required*

- Hazard Communication
- Hazardous Waste Management
- Glove Box Training
- Basic Safety Training
- Other: Ethidium Bromide Training

**Additional training requirements**

*List additional, local training requirements.*

**12. Approval**

*Standard operating procedures must be approved by the laboratory manager and directorate safety coordinator.*



Laboratory manager (*name, signature, date*): \_\_\_\_\_

Directorate safety coordinator (*name, signature, date*): \_\_\_\_\_

**Additional approvals**

*List subject matter experts consulted for approval:*

Person consulted

Person consulted

**Additional prior approvals required**

*List any tasks that require prior approval by the principal investigator or laboratory manager (for example, use of restricted chemicals and other higher hazard chemicals and running of higher hazard operations):*

Task requiring prior approval

Task requiring prior approval

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