Standard Operating Procedure

**Ethidium Bromide (EtBr)**

Print a copy and insert into your   
*Laboratory Safety Manual* and *Chemical Hygiene Plan*.   
Refer to instructions for assistance.

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| **Department:** | Chemistry & Biochemistry |
| **Date SOP was written:** | 1/15/2013 |
| **Date SOP was approved by PI/lab supervisor:** | 1/15/2013 |
| **Principal Investigator:** | Irene Chen |
| **Internal Lab Safety Coordinator/Lab Manager:** | Irene Chen |
| **Lab Phone:** | 805-893-8085 |
| **Office Phone:** | 805-893-8364 |
| **Emergency Contact:** | Irene Chen, 617-710-8741 (cell) |
| *(Name and Phone Number)* |
| **Location(s) covered by this SOP:** | *Chemistry 1142* |
| *(Building/Room Number)* |

**Type of SOP:**  Process Hazardous Chemical  Hazardous Class

**Purpose**

Ethidium bromide (EtBr) is a ‘Mutagen’. If not stored and handled properly, this can pose a serious threat to the health and safety of laboratory personnel, emergency responders and chemical waste handlers. Hence, it is important to follow safety protocols to handle this chemical.

Ethidium Bromide is commonly used as a non-radioactive DNA and RNA stain to identify and visualize nucleic acid bands in electrophoresis and perform other methods of nucleic acid separation. Solutions of EtBr fluoresce readily with a reddish-brown color when exposed to ultraviolet (UV) light. Although it is an effective tool for genomic research, its hazardous properties require special safe handling and disposal.

**Physical & Chemical Properties/Definition of Chemical Group**

CAS#: 1239-45-8

Class: Reproductive Toxin

Molecular Formula: C21H20BrN3

Form (physical state): powder

Color: Dark Red

Boiling point: Decomposes. (261°C or 501.8°F)

**Potential Hazards/Toxicity**

EtBr is a mutagen (may cause genetic damage) and is moderately toxic after an acute exposure.

* EtBr can be absorbed through skin, and will stain it purple.
* EtBr is an irritant to the skin, eyes, mouth, and upper respiratory tract.
* Some *alternative stains* are less mutagenic and less toxic than EtBr. If the toxicological data is lacking or unclear, handle the stain in the same way as EtBr.
* Some alternative stains are suspended in dimethyl sulfoxide (DMSO), which can increase skin absorption of organic compounds.

**Personal Protective Equipment (PPE)**

**Respiratory Protection**

Respirators should be used only under any of the following circumstances:

* As a last line of defense (i.e., after engineering and administrative controls have been exhausted).
* When Permissible Exposure Limit (PEL) has exceeded or when there is a possibility that PEL will be exceeded.
* Regulations require the use of a respirator.
* An employer requires the use of a respirator.
* There is potential for harmful exposure due to an atmospheric contaminant (in the absence of PEL)
* As PPE in the event of a chemical spill clean-up process

Lab personnel intending to use/wear a respirator mask must be trained and fit-tested by EH&S. This is a regulatory requirement. (<http://map.ais.ucla.edu/go/1004655>)

**Hand Protection**

Handle with nitrile gloves. Gloves must be inspected prior to use. Use proper glove removal technique (without touching glove's outer surface) to avoid skin contact with this product. Dispose of contaminated gloves after use in accordance with applicable laws and good laboratory practices. Wash and dry hands.

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

Refer to glove selection chart from the links below:

<http://www.ansellpro.com/download/Ansell_8thEditionChemicalResistanceGuide.pdf>

OR

<http://www.allsafetyproducts.biz/page/74172>

OR

<http://www.showabestglove.com/site/default.aspx>

OR

<http://www.mapaglove.com/>

**Eye Protection**

ANSI approved safety glasses or chemical splash goggles at a minimum should be worn.

**Skin and Body Protection**

Lab Coat must be worn at all times. Full length pants. Closed toe shoes.

**Hygiene Measures**

Avoid contact with skin, eyes and clothing. Wash hands before breaks and immediately after handling the product.

**Engineering Controls**

Always handle ethidium bromide in a certified chemical fume hood. Use dry materials in a fume hood, or choose premixed solutions to avoid inhalation exposure.

**First Aid Procedures**

**If inhaled**

In the case of EtBr ingestion, obtain medical attention immediately. If EtBr dust is inhaled, move the victim to a source of fresh air.

**In case of skin contact**

In the event of skin exposure, remove contaminated clothing and immediately wash the affected area with soap and copious amounts of water for 15 minutes.

**In case of eye contact**

Immediately flush eyes with copious amounts of water for at least 15 minutes, preferably in an emergency eyewash.

**If swallowed**

In the case of EtBr ingestion, obtain medical attention immediately. If EtBr dust is inhaled, move the victim to a source of fresh air.

**Special Handling and Storage Requirements**

All work with ethidium bromide is to be done in an "ethidium bromide" designated area in order to keep ethidium bromide contamination to a minimum. Any persons in this area are required to wear personal protective equipment. Safety shower and eye wash stations should be easily accessible where ethidium bromide is used. Persons operating gel system are to take added caution when using ultraviolet light to visualize gels. Persons are to make sure the UV light is off before they open the UV box and that the UV light is turned off when they are finished. Avoid exposing unprotected skin and eyes to intense UV sources.

**Spill and Accident Procedure**

**Chemical Spill Dial 911 and call EH&S at 893-3194**

**Spill** – Assess the extent of danger. Help contaminated or injured persons. Evacuate the spill area. Avoid breathing vapors. If possible, confine the spill to a small area using a spill kit or absorbent material. Keep others from entering contaminated area (e.g., use caution tape, barriers, etc.).

**Small (<1 L)** – If you have training, you may assist in the clean-up effort. Use appropriate personal protective equipment and clean-up material for chemical spilled. Double bag spill waste in clear plastic bags, label and take to the next chemical waste pick-up.

**Large (>1 L)** – Dial **911** (or 310-825-1491 from cell phone) and EH&S at **893-3194** for assistance.

**Chemical Spill on Body or Clothes** – Remove clothing and rinse body thoroughly in emergency shower for at least 15 minutes. Seek medical attention. *Notify supervisor and EH&S at x59797 immediately.*

**Chemical Splash Into Eyes** – Immediately rinse eyeball and inner surface of eyelid with water from the emergency eyewash station for 15 minutes by forcibly holding the eye open. Seek medical attention. *Notify supervisor and EH&S at* **893-3194** *immediately.*

# **Medical Emergency Dial 9-911**

**Life Threatening Emergency:** Dial 9-**911** Contact PI and EH&S as soon as practical. *Note: All serious injuries must be reported to EH&S at x3194 within 8 hours.*

**Non-Life Threatening Emergency** – Go to Student Health, building 588, **x5361,** Hours: M, T, R, F 8 a.m. to 4:30 p.m, W 9 a.m. to 4:30 pm and R 5 to 7 p.m. by appointment. At all other times report to Goleta Valley Cottage Hospital (emergency room) at 351 South Patterson Avenue, **805-967-3411**. Contact PI and EH&S as soon as practical.*Note: All serious injuries must be reported to EH&S at x3194 within 8 hours.*

**Decontamination/Waste Disposal Procedure**

Waste disposal procedures

1. All solid ethidium bromide contaminated waste shall be disposed of into waste containers specifically designated for ethidium bromide waste. Examples of solid ethidium bromide waste material include gloves, pipette tips, paper towels, and electrophoretic gels.
2. Once the waste container is full, dispose of according the EH&S hazardous waste guidelines.

Decontamination of Equipment

Equipment that needs to be decontaminated (for repair or change of location etc.) must be placed in a mixture of one part bleach, one part soap and one part water. Let the equipment soak for a couple of hours and then wash and rinse equipment with copious amounts of water.

*General hazardous waste disposal guidelines:*

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**Label Waste**

* Affix a hazardous waste tag on all waste containers as soon as the first drop of waste is added to the container

**Store Waste**

* Store hazardous waste in closed containers, in secondary containment and in a designated location
* Double-bag dry waste using transparent bags
* Waste must be under the control of the person generating & disposing of it

**Dispose of Waste**

* Dispose of regularly generated chemical waste within 90 days
* Call EH&S for questions and for pick-up
* Empty Containers: Dispose as hazardous waste irrespective of the container size

Prepare for transport to pick-up location

* Check waste tag
* Use secondary containment

**Safety Data Sheet (SDS) Location**

Online SDS can be accessed at <http://www.chem.ucsb.edu/about/safety> .

**Protocol/Procedure**

Ethidium Bromide is used to stain DNA and RNA, particularly in gels. We purchase EtBr in solution, to minimize contact with powder. Be aware that EtBr migrates into the buffer during electrophoresis, so the buffer is contaminated after the gel is completed. Always clean up spills promptly to avoid dust formation. Also minimize use of EtBr as other safer alternatives (e.g., Sybr Safe) exist. Dispose of any waste that comes into contact with EtBr as outlined. Decontamination can also be achieved using charcoal (for example, see Sigma-Aldrich’s GREEN ethidium bromide extraction kit) – use this method if you plan to generate a lot of waste (e.g., buffer containing EtBr). Keep plasticware that has been in contact with EtBr separate from other plasticware – this is also an issue because EtBr (and similar dyes) leaches into and out of the plasticware, so be aware of this if you do not want EtBr in your experiment.

**NOTE**

Any deviation from this SOP requires approval from PI.

**Documentation of Training** (signature of all users is required)

* Prior to conducting any work with ethidium bromide (EtBr), designated personnel must provide training to his/her laboratory personnel specific to the hazards involved in working with this substance, work area decontamination, and emergency procedures.
* The Principal Investigator must provide his/her laboratory personnel with a copy of this SOP and a copy of the SDS provided by the manufacturer.
* The Principal Investigator must ensure that his/her laboratory personnel have attended appropriate laboratory safety training or refresher training within the last one year.

I have read and understand the content of this SOP:

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| **Name** | **Signature** | **Date** |
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