Standard Operating Procedure

**Ethidium Bromide (EtBr)**

*This is an SOP template and is not complete until: 1) lab specific information is entered into the box below 2) lab specific protocol/procedure is added to the protocol/procedure section and
3) SOP has been signed and dated by the PI and relevant lab personnel.*

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

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| **Department:** | Click here to enter text. |
| **Date SOP was written:** | Click here to enter a date. |
| **Date SOP was approved by PI/lab supervisor:** | Click here to enter a date. |
| **Principal Investigator:** | Click here to enter text. |
| **Internal Lab Safety Coordinator/Lab Manager:** | Click here to enter text. |
| **Lab Phone:** | Click here to enter text. |
| **Office Phone:** | Click here to enter text. |
| **Emergency Contact:** | Click here to enter text. |
| *(Name and Phone Number)* |
| **Location(s) covered by this SOP:** | Click here to enter text. |
| *(Building/Room Number)* |

**Type of SOP:** [ ]  Process [x] 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 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 x59797**

**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 x59797 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 x59797 immediately.*

# **Medical Emergency Dial 911 or x52111**

**Life Threatening Emergency, After Hours, Weekends And Holidays** – Dial **911** (or 310-825-1491 from cell phone) or contact the Ronald Reagan UCLA Medical Center (emergency room) directly at **x52111** (located at 757 Westwood Plaza, enter from Gayley Avenue). *Note: All serious injuries must be reported to EH&S at* ***x59797*** *within 8 hours.*

**Non-Life Threatening Emergency** – Go to the Occupational Health Facility (OHF), **x56771,** CHS room 67-120(This is on the 6th floor, 7th corridor, room 120. Enter through the School of Dentistry on Tiverton Drive and proceed to the “O” elevator to the 6th floor.)Hours: M - F, 7:30 a.m. to 4:30 p.m. At all other times report to Ronald Regan UCLA Medical Center (emergency room) at **x52111**. *Note: All serious injuries must be reported to EH&S at x59797 within 8 hours.*

**Needle stick/puncture** **exposure** (as applicable to chemical handling procedure) – Wash the affected area with antiseptic soap and warm water for 15 minutes. For mucous membrane exposure, flush the affected area for 15 minutes using an eyewash station. Page the needle stick nurse by dialing **231** from a campus phone, enter **93333** when prompted and then enter your extension. Hours: M – F, 8:00 a.m. to 4:00 p.m. At all other times report to Ronald Regan UCLA Medical Center (emergency room) at **x52111**. *Note: All needle stick/puncture exposures must be reported to EH&S at x59797 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 UCLA 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:*

**Label Waste**

* Affix an on-line hazardous waste tag on all waste containers using the Online Tag Program <http://otp.ucop.edu/> 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 <http://map.ais.ucla.edu/go/1002774>
* 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 at x61887 for questions
* Empty Containers
* Dispose as hazardous waste if it once held extremely hazardous waste (irrespective of the container size) <http://ehs.ucla.edu/Pub/ExtremelyHazardousWaste.pdf>
* Consult waste pick-up schedule <http://ehs.ucla.edu/pub/HazWaste%20Pickup%20Schedule.pdf>

Prepare for transport to pick-up location

* Check on-line waste tag
* Write date of pick-up on the waste tag
* Use secondary containment

**Safety Data Sheet (SDS) Location**

Online SDS can be accessed at [http://msds.ehs.ucla.edu](http://msds.ehs.ucla.edu/).

**Protocol/Procedure (Add lab specific Protocol/Procedure here)**

Click here to enter text.

**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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