Standard Operating Procedure for Laboratory Processes

A Standard Operating Procedure (SOP) is a written set of instructions that document how to safely perform work involving hazardous materials or hazardous operations. Print out the completed form and keep a readily accessible hard copy in the lab (also keeping an electronic copy is highly recommended).

**Chemical Name or Process:**

Making or working with solutions containing ethidium bromide in a laboratory setting.

**Purpose:** Ethidium Bromide (EtBr) is a common non-radioactive stain used for the identification and visualization of Nucleic acids in electrophoresis and other methods of nucleic acid separations. Gels, etc, stained with EtBr fluoresce readily with a reddish-brown color under UV light. This fluorescence increases when EtBr is bound to the double-stranded DNA.

**Potential Hazards/Toxicity:** Ethidium Bromide is considered moderately toxic chemical, but a potent mutagen (reasonably expected to cause genetic damage). However, it is not flammable, reactive, or corrosive. Its mutagenicity is theorized to be a result of its bonding to the double stranded DNA, thereby deforming the molecule. Ethidium Bromide can be absorbed through skin, staining it purple. The gravest danger is the inhalation of solid EtBr as a dust or an “aerosolized” EtBr solution. Exposure is to be avoided at all times by performing solid or aerosolized EtBr work in a working fume hood.

For solid EtBr:

Oral LD50 (rat) = 1,503mg/kg,

Inhalation LC50 (rat) = 0.118-0.134 mg/L/6H.

EtBr is listed as an irritant via all exposure routes.

It is not listed by ACGIH, IARC, NTP, or CA prop 65 as carcinogenic.

Exposure Symptoms:An acute exposure to ethidium bromide causes irritation of the mouth, upper respiratory tract, skin, and eyes.

**Engineering Controls:**

Exposure is to be avoided at all times by performing solid or aerosolized EtBr work in a working fume hood.

**Personal Protective Equipment (PPE)-**

**Hand Protection:**

Nitrile gloves provide excellent protection for ethidium bromide. Wash hands thoroughly after removing gloves.

NOTE: Consult with your preferred glove manufacturer, the (M)SDS and other sources to ensure that the gloves you plan on using are compatible with chemical(s) being used.

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 :**

Wear appropriate eye protection at all times, safety glasses at a minimum. Goggles are required whenever there is a potential for a hazardous liquid splash, as per the Chemical Hygiene Plan Sec 3.1.b

**Skin and Body Protection:**

Lab personnel working with the chemicals need to wear full-length pants or its equivalent, closed-toe footwear with no skin being exposed, and a lab coat. Remove lab coat and gloves when leaving the lab.

**Hygiene Measures:**

Wash hands after working with the hazardous substances and when leaving the lab/shop.

**Respirators may be required 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) will or may be exceeded or the airborne concentration is unknown.
* Regulations require 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

Prior to obtaining a respirator, an exposure assessment of the process or procedure must be conducted. If respiratory protection is required, then lab personnel must obtain respiratory protection training, a medical evaluation, and a respirator fit test through EH&S. This is a regulatory requirement.

**First Aid Procedures for Chemical Exposures**

**If inhaled:**

If breathed in, move person into fresh air. If not breathing, give artificial respiration. Consult a physician.

**In case of skin contact:**

In case of contact, immediately flush skin with plenty of water for at least 15 minutes while removing contaminated clothing and shoes. Cold water may be used. Wash clothing before reuse. Thoroughly clean shoes before reuse. Get medical attention, as necessary.

**In case of eye contact:**

Immediately flush eyes with plenty of water for at least 15 minutes. Check for and remove any contact lenses.

**If swallowed:**  Never give anything by mouth to an unconscious person. Rinse mouth with water. Consult a physician.

**Special Handling and Storage Requirements**

Keep container tightly closed in a dry and well-ventilated place. Keep in a dry place. Storage class: very toxic hazardous materials.

**Spill and Accident Procedure**

**Chemical Spill Dial 911 and 756-6661**

**Spill** – Assess the extent of danger. Help contaminated or injured persons. Evacuate the spill area. Avoid breathing vapors. If safe, 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. Double bag spill waste in plastic bags, label and arrange hazardous waste pick-up.

**Large (>1 L)** – Evacuate spill area. Dial **911** and EH&S at 756-6661 for assistance.Remain available in a safe, nearby location for emergency personnel.

**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, advisor or P.I. immediately.*

**Chemical Splash Into Eyes** – Immediately rinse eyeball and inner surface of eyelid with water from the emergency eyewash station fora minimum of 15 minutes by forcibly holding the eye open. Seek medical attention. *Notify supervisor, advisor or P.I. immediately.*

# **Medical Emergency Dial 911 or 756-6661**

**Life Threatening Emergency, After Hours, Weekends And Holidays** – Dial 911

*Note: All serious injuries must be reported to Supervisor/PI within 8 hours. Note: Any and all loss of consciousness requires a 911 call*

**Non-Life Threatening Emergency** –

* Students: Seek medical attention at the campus Health Center **M, T, Thu, Fr 8:00 am – 4:30 pm and W 9:00 am – 4:30 pm**
* Emergency Medical services in the community are available at any time at hospital emergency rooms and some emergency care facilities.

***All injuries must be reported to PI/Supervisor immediately and follow campus injury reporting. Follow procedures for reporting of student, visitor injury on the EH&S website at:*** <http://afd.calpoly.edu/riskmgmt/incidentreporting.asp>

* Paid staff, students, faculty: seek initial medical attention for all non-life threatening injuries at:
  + MED STOP, 283 Madonna Road, Suite B (next to See's Candy in Madonna Plaza)  
    (805) 549-8880 Hours: M-F 8a - 8p; Sat/Sun 8a - 4p
  + **After MED Stop Hours:** Sierra Vista Hospital Emergency Room   
    1010 Murray Avenue (805) 546-7651, Open 24 hours

***All injuries must be reported to PI/Supervisor immediately and follow campus injury reporting for employee injuries (Workmen’s Comp.). Follow procedures on the EH&S website at:*** [***http://afd.calpoly.edu/riskmgmt/incidentreporting.asp***](http://afd.calpoly.edu/riskmgmt/incidentreporting.asp)

**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. Seek medical attention. *Note: All needle stick/puncture exposures must be reported to supervisor, advisor or P.I. and EH&S office immediately.*

**Decontamination/Waste Disposal Procedure**

**General hazardous waste disposal guidelines: Ethidium bromide is a hazardous waste. All solutions and solids containing ethidium bromide will follow the instructions below:**

**Label Waste**

* Affix a hazardous waste tag on all waste containers as soon as the first drop of waste is added to the container. Generic waste labels can be found here: <http://afd.calpoly.edu/ehs/docs/hazwaste_label_template.pdf>

**Store Waste**

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

**Dispose of Waste**

* Dispose of regularly generated chemical waste as per guidelines on EH&S website at: <http://afd.calpoly.edu/ehs/docs/csb_no6.pdf>
* Prepare for transport for pick-up. Use secondary containment.

Call EH&S at 756-6661 for questions.

**Empty Containers-**

* Dispose as hazardous waste if container once held extremely hazardous waste (irrespective of the container size) A list can be found at: <http://afd.calpoly.edu/ehs/docs/extremely_hazardous_wastes.pdf>
* All other containers are legally empty once a concerted effort is made to remove, pour out, scrape out, or otherwise completely empty the vessel. These may be disposed of as recycling or common trash as appropriate.

**Safety Data Sheet (SDS) Location**

Online SDS can be accessed at MSDSOnline: <http://hq.msdsonline.com/csuedusl/Search/Default.aspx>

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

Click here to enter step by step procedure here.

**NOTE:**

Any deviation from this SOP requires approval from PI.

**Date:** Click here to enter a date. **P.I. or Supervisor:** Click here to enter name.

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

* The Principal Investigator must ensure that his/her laboratory personnel have attended appropriate laboratory safety training or refresher training within the last one year.
* Training must be administered by PI or Lab Manager to all personnel in lab prior to start

of work with particularly hazardous substance or newly synthetic chemical listed in the

SOP.

* Refresher training will need to be provided when there is a change to the work

procedure, an accident occurs, or repeat non-compliance.

I have read and understand the content, requirements, and responsibilities of this SOP:

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