

Standard Operating Procedure

Ethidium Bromide

Print a copy of this SOP and insert into your Safety Binder.

- SOP Information

Department:	CSUDH – (Name of your Department)		
Date SOP was written:	Click or tap to enter a date.		
Date SOP was approved by PI/lab supervisor:	Click or tap to enter a date.		
Principal Investigator:	Click or tap here to enter text.		
Chemical Hygiene Officer /Lab Manager:	Ricardo Magallanes/		
Lab Phone:	(XXX) – XXX – XXXX		
Office Phone:	(XXX) – XXX – XXXX		
Emergency Contact:	EHS (310) 243 – 3000		
Emergency contact.	(Name and Phone Number)		
Location(s) covered by	Campus		
this SOP:	(Building/Room Number)		
SOP Specific lab procedure or experiment	[⊠] Generic use of specific chemical or class of chemicals w/ similar hazards [□] Generic use of equipment		

Contents

-	SOP Information	i
1.	Purpose	1
2.	Subject Chemicals Used in this Laboratory	1
3.	Properties & Hazards	1
4.	Administrative Control	1
5.	Engineering Controls	1
6.	Personal Protective Equipment	2
7.	Special Handling & Storage Requirements	3
8.	First Aid	3
9.	Medical Emergency	4
10.	Spill & Accident Procedures	4
11.	Decontamination & Waste Disposal Procedure	5
12.	Safety Data Sheet (SDS) Location	5
13.	Required Travel/Approvals	6
14.	Additional Notes	
15.	Documentation of Training	7
16.	Lab Specific Procedures	

Definitions

- **P.I.** Principal Investigator. Usually a professor in charge of a laboratory or set of laboratories who is actively undertaking research. They are considered the front-line supervisor and are responsible for training and personnel safety in the laboratory.
- **SDS** Safety Data Sheets. An essential component of the GHS and are intended to provide comprehensive information about a substance or mixture for use in workplace chemical management. Also Known as MSDS (Material Safety Data Sheet)
- **S.O.P.** Standard Operating Procedure. A written set of instructions that document how to safely perform work involving hazardous chemicals or hazardous operations. Includes training documentation.

1. Purpose

This document covers basic chemical safety information for ethidium bromide. The use of ethidium bromide is subject to pre-approval by the Principal Investigator (PI) and/or Supervisor.

2. Subject Chemicals Used in this Laboratory

DO NOT USE ETHIDIUM BROMIDE UNTIL YOU HAVE OBTAINED THE NECESSARY PREAPPROVAL.

3. Properties & Hazards

Ethidium bromide is a commonly used chemical for the visualization of DNA in gel electrophoresis. It is known to intercalate into DNA and thought to cause reproductive harm and possibly act as a mutagen. Ethidium bromide powder is also highly toxic by inhalation.



4. Administrative Control

In addition to the practices described below, follow procedures as specified in the lab-specific and special handling/use sections of this SOP.

General practices:

- 1. Be sure to review the Safety Data Sheet (SDS) for all chemicals to be used in the experiment.
- 2. **Never work alone.** At least one other person must be present in the same laboratory when any work involving hazardous chemicals is being done.
- 3. Eliminate or substitute for a less hazardous material when possible.
- 4. Design your experiment to use the least amount of material possible to achieve the desired result.
- 5. Verify your experimental set-up and procedure prior to use. Be familiar with the Safety Data Sheets for all chemicals in use. Assess the hazards to ensure that appropriate controls are in place to minimize risk and address emergency shut-down procedures as appropriate.
- 6. Consult with the **P.I.** if the work involves procedure scale-up or other large quantities or there are any questions regarding appropriate safety procedures.

5. Engineering Controls

In addition to the practices described below, follow procedures as specified in the lab-specific and special handling/use sections of this SOP.

General practices:

- 1. In general, it is preferable to perform all work with hazardous chemicals in a fume hood. Sash height should be kept as low as possible to avoid the escape of vapors, gases and particulates.
 - Use a fume hood when working with materials which are toxic by inhalation.
- 2. Supplemental equipment such as blast shields should be used when working with chemicals or processes that may result in explosions or pressure releases.
- 3. Consider the use of a glove box, toxic gas cabinet or other local exhaust in order to further contain hazards as appropriate.

6. Personal Protective Equipment

In addition to the practices described below, follow procedures as specified in the lab-specific and special handling/use sections of this SOP.

Respiratory Protection

Respiratory protection is generally not required for lab research, provided the appropriate engineering controls are employed. Respirators should be used only under any of the following circumstances:

Lab personnel intending to use/wear a respirator mask must be trained and fit-tested by EH&S. This is a regulatory requirement. If you think that your process may require respirator use, contact EH&S for assistance

Hand Protection

Disposable nitrile gloves provide sufficient protection for most routine lab operations involving small quantities. They should be changed if liquid is splashed onto them. They are not appropriate for longer operations or operations using larger quantities.

For longer operations, or operations using larger quantities, use thicker gloves made from a material appropriate for the specific chemical in use (e.g., natural rubber, butyl, neoprene, nitrile, PVA). When working chemicals or processes that increase the risk of exposure to fire, use hand protection appropriate to both the risk of chemical exposure and the risk from fire. Gloves must be inspected prior to use for signs of wear or damage. Such gloves should be disposed of in accordance with appropriate laboratory disposal practices.

Use proper glove removal technique (without touching glove's outer surface) to avoid skin contact with any chemical residues on the surface. Wash and dry hands after use.

For additional information on selection of glove material, review the specific chemical Safety Data Sheet. Consult with your preferred glove manufacturer's website to ensure that the gloves you plan on using are compatible with a specific chemical substance. Common manufacturer glove selection guidance can be found at:

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

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

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

http://www.mapa-pro.com/our-gloves/protections/chemical-protection/b/handled_product.html

Eye Protection

Use safety glasses with side shields or tightly fitting safety goggles whenever working in the laboratory.

Skin and Body Protection

Long pants, closed toed-shoes, shirt and a lab coat must be worn whenever working in the laboratory. Flame resistant Nomex® lab coats should be used when working with chemicals or processes that increase the risk of fire. Fully extend sleeves to the wrists and keep buttoned at all times. Avoid wearing synthetic clothing when practicable.

Hygiene Measures

Wash hands immediately and thoroughly after handling chemicals. Any contaminated clothing should be disposed of or washed before reuse.







7. Special Handling & Storage Requirements

ALL OPERATIONS involving powder or mists of ethidium bromide must be done in a fume hood.

Liquid: Store in dark and cold area, preferably in a plastic container; away from strong oxidizing agents Solid: Store in designated areas.

8. First Aid

In addition to the practices described below, follow procedures as specified in the lab-specific and special handling/use sections of this SOP.

Consult the Safety Data Sheet for the subject chemical for specific first aid procedures. General first aid procedures for hazardous chemicals are provided below.

If inhaled

Move to fresh air. Have victim rest in half-upright position. Artificial respiration victim is not breathing. Seek medical attention immediately.

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. Wash clothing before reuse. Thoroughly clean shoes before reuse. Get medical attention immediately

In case of eye contact

Check for and remove any contact lenses. In case of contact, immediately flush eyes with plenty of water from emergency eyewash station for at least 15 minutes. Get medical attention immediately.

If swallowed

If swallowed, do not induce vomiting unless directed to do so by medical personnel. Never give anything by mouth to an unconscious person. Loosen tight clothing such as a collar, tie, belt or waistband. Get medical attention immediately.

9. Medical Emergency

Be familiar with information in the CSUDH Emergency poster.

- **a. Life Threatening Emergency** (all times: Business Hours, After Hours, Weekends and Holidays)--CALL 911 if the condition is LIFE THREATENING or REQUIRES IMMEDIATE MEDICAL ATTENTION.

 Note: All serious injuries must be reported to EH&S at ext. 2895 within 8 hours. Complete online incident report at https://www.csudh.edu/Assets/csudh-sites/rm-ehos/docs/risk-management-ehos/accident-report-form-std-268.pdf
- **b. Non-Life Threatening Emergency** Notify your supervisor or faculty staff if condition is not life threatening or does not require immediate medical attention.

ALL WORK RELATED INJURIES MUST BE REPORTED via the On-line Incident Form https://www.csudh.edu/hr/workers-compensation/ or call Human Resources, Workers Compensation (310) 243-3771.

10. Spill & Accident Procedures

In addition to the practices described below, follow procedures as specified in the lab-specific and special handling/use sections of this SOP.

Evacuate the spill area. Post someone or mark-off the hazardous area with tape and warning signs to keep other people from entering the area. Keep the appropriate fire extinguisher nearby. Avoid incompatible extinguishing agents. Use Class A-B-C or B-C for flammable liquids. **Fire extinguishers containing water are not suitable for flammable liquid fires.**

Spill – Assess the extent of danger. Help contaminated or injured persons if safe to do so. 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, <100 g) – 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, >100 g) - Dial 911 and EH&S at ext. 3000 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* **ext. 3000** *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* **ext. 3000** *immediately.*

11. Decontamination & Waste Disposal Procedure

In addition to the practices described below, follow procedures as specified in the lab-specific and special handling/use sections of this SOP.

All of the subject chemicals must be disposed as a hazardous waste.

Label Waste

Hazardous waste labels must be placed on the hazardous waste container upon the start of
accumulation. Labels identifying the material as Reproductive Toxicant must appear on the
bottles and secondary containers. Ethidium bromide powder must also be identified as an Acute
Toxicant. Also, if not plainly visible (e.g., through a cabinet window), labelling must be applied to
storage locations where these are stored to avoid an inadvertent encounter.

Store Waste

- Hazardous waste containers must be kept closed, except when adding waste.
- Hazardous waste containers must be stored in secondary containment to adequately contain all
 of the contents of the container.
- Hazardous waste containers must be inspected weekly for signs of leaks, corrosion, or deterioration.
- Also, if not plainly visible (e.g., through a cabinet window), labelling must be applied to storage locations where these are stored to avoid an inadvertent encounter.

Dispose of Waste

- Hazardous waste must be transferred to EH&S for disposal within 6 months of being generated.
- Empty Containers: At no time should full or partially full containers be placed in the trash.
- Hazardous Waste Disposal:
 - o Visit: https://www.csudh.edu/ehs/environmental/hazardous-waste
 - o Fill out the "CSUDH Hazardous Waste Label"
 - EH&S will pick up your waste within 1-3 days.
- **DO NOT** dispose of chemicals by pouring them down the drain or placing them in the trash.
- **DO NOT** use fume hoods to evaporate chemicals.

Decontamination

• Deactivation with hypophosphorous acid solution is the most-effective means of decontamination. See. Lunn, G. and Sansone, E.B. Appl Ind Hyg 1989, 4 (9), 234-237.

Refer to the CSUDH Chemical Hygiene Plan for more information on waste management.

12. Safety Data Sheet (SDS) Location

Online SDSs can be accessed at http://hq.msdsonline.com/csuedusl

.

13. Required Travel/Approvals

In addition to the practices described below, follow procedures as specified in the lab-specific and special handling/use sections of this SOP.

All work with the subject chemical (s) requires the following prior to beginning work:

- Must be pre-approved by the Principal Investigator prior to use and all training must be well documented.
- 2. Must be familiar with the CSUDH Chemical Hygiene Plan. https://www.csudh.edu/ehs/health-safety-programs-policies/
- 3. Must have documented Laboratory Safety training.
- 4. Must read the relevant Safety Data Sheet (formerly referenced as Material Safety Data Sheets).
- 5. Any additional laboratory specific training that is needed is referenced in the 'Laboratory Specific Use Procedures' section. Signed and dated training documents must be uploaded into each assigned researchers' training records.

14. Additional Notes

Any deviation from this SOP requires approval from P.I.

15. Documentation of Training

- Prior to conducting any work with the subject chemicals, designated personnel must provide training to his/her laboratory personnel specific to the hazards and procedures involved in working with these substances.
- 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:

Name	Signature	CSUDH ID#	Date

16. Lab Specific Procedures

The following describe how the subject chemicals are used in this laboratory beyond the practices described above.

This section must describe lab-specific procedures to address the safe use of all highly hazardous chemicals from this band in use in the laboratory. These procedures may be organized around specific chemicals, specific tasks or the band as a whole. The following minimum requirements must be met:

- · Identify designated use areas within the laboratory for highly hazardous chemicals
- Identify maximum use quantities for which the procedures in this band apply.
- If it is determined that this SOP is sufficient to address the safe use of subject chemical in this lab, then include the following statement in this section: "Procedures described in this SOP are sufficient for addressing the safe use of subject chemical in this laboratory within the listed quantity limitations."
- If it is determined that this SOP is not sufficient to address the safe use of a chemical from the lab, then write lab-specific procedures for to address these high hazard operations. Such operations are generally indicated by:
 - o tasks requiring the use of specialized PPE,
 - o tasks using highly hazardous chemicals outside of the fume hood,
 - o tasks using larger quantities of hazardous chemicals,
 - tasks involving the use of particular chemicals considered by CSUDH EHS to be extremely hazardous, and
 - o tasks considered to present high risk by lab personnel.

A few examples of what lab-specific tasks may look like are provided below:

Task #1: Title of the specific procedure being done.

- 1) Provide step-by-step instructions in a numbered/lettered format.
- 2) Include in the procedure any relevant:
 - a) Locations of "designated areas" as called for in the special handling section of the SOP, or as otherwise required by regulations. The entire laboratory, fume hood, or a portion of the laboratory may be considered as a designated area.
 - b) Use of specific administrative, engineering and PPE controls.
 - c) Specific quantity use limits/restrictions.
 - d) Specific storage requirements.
 - e) Specific first aid and spill procedures (including what should be handled by whom).
 - f) Specific disposal procedures.
 - g) Process-specific PI approvals required.

Task #2: Making dilutions of the acids and bases.

- 1) Consult with PI and obtain approval if quantities greater than 4 L are needed.
- 2) In a fume hood, add the appropriate amount of concentrated acid or base to the calculated amount of water.
- 3) Return the concentrated acids/bases to the proper secondary containment or cabinet.

Task #3: Using the pH meter.

- 1) Calibrate on the day of pH testing using at least 2 standards.
- 2) Before use, rinse the electrode with deionized water and blot dry with a kim-wipe.
- 3) Transfer the electrode to the test solution.
- 4) If using a stir plate, make sure the electrode does not touch the stir bar.

- 5) Record the pH when the reading is stable (5–20 seconds after insertion of the electrode into the solution)
- 6) Add dilute acid or dilute base drop-wise until the correct pH is reached.
- 7) Rinse the electrode with deionized water and store according to the manufacturer's instructions.
- 8) Make sure the acid and base caps are on tightly.

Add as many tasks as necessary.