Standard Operating Procedure

Date:

SOP Title: Ethidium Bromide in Agarose Gel

Principal Investigator:

Room and Building:

Lab Phone Number:

1. Process

**All work with Ethidium Bromide (EtBr) is confined to the gel running and pouring benches and the gel doc areas (which are labeled).**

**When you leave the Ethidium Bromide area you MUST remove your gloves to prevent EtBr being transferred to other parts of the lab.**

* Do all of these steps using nitrile gloves!!
* Measure out agarose powder and add appropriate amount of1x TAE buffer
* Boil until all agarose has been dissolved and allow to cool until warm to touch.
* Add 4ul of EtBr stock solution directly to the get tray in the fume hood and then add 50ml of liquid agarose using filter tip. Mix the EtBr into the gel by rocking the tray gently.
* Dispose of filter tip in EtBr waste bucket located in the fume hood.
* Use Gel Doc system to visualize gel. Do not drip buffer when transporting the gel!
* Dispose of EtBr gel in drying tray in the fume hood.
1. Hazardous Chemicals

Ethidium Bromide, (Et Br) is a mutagen and must be handled carefully, Read a MSDS from manufacturer and EH&S fact sheet on DNA stains: <https://www.sigmaaldrich.com/US/en/sds/sigma/e1510>

1. Potential Hazards
* Mutagen (may cause genetic damage) at high and low concentrations
* Toxic when working with stock solutions, powder or crystals.
* **Avoid working with Ethidium Bromide in the raw form** (powder or crystal). Order pre-diluted stock solutions.
* Irritant to the skin, eyes, mucous membranes, and upper respiratory tract when working with stock solutions and powders.
1. Approvals Required
2. Designated Area
* Use EtBr in a designated area, this area is labeled. When working in this area wear gloves and wash hands before eating.
* EtBr stock solution is located in room \_\_\_ in the chemical container. Two 1 mL aliquots are provided in the Ethidium Bromide working area.
* Ethedium Bromide powder is Not used in this lab as we use premixed solutions.
1. Special Handling Procedures and Storage Requirements
* Recommend wearing safety glasses and gloves when handling gels and buffer solutions.
* Short wave ultraviolet radiation will harm your eyes and skin therefore make sure the gel doc is closed before turning UV on (note there is safety system to help prevent this from happening but it is good practice not to do it anyway).
* EtBr stock solutions and powder should be stored away from strong oxidizing agents in a cool, dry place and the container must be kept undamaged and tightly closed.
1. Personal Protective Equipment
* Wear nitrile gloves, along with Lab coat and safety goggles which are provided in room ­­­­­\_\_\_\_\_.
* When dealing with Ethidium Bromide also use the fume hood when possible.
1. Engineering/Ventilation Controls
2. Spill and Accident Procedures
* Put on heavy duty gloves (preferred, since abrasive pad may puncture thin nitrile gloves) or double layers of nitrile gloves.
* Soak the spill up with paper towels or some other absorbent and place absorbents in a secondary container that does not leak.
* Once the area is dry, spray the affected area with decontamination solution (10% bleach solution or 1% sodium hypochlorite solution) and allow it to sit for at least 10 minutes.
* Using paper towels dry up the area and then wipe the area down with absorbents dipped in tap water. Repeat this process until the area is clean.
* Using a UV light, check the area to ensure that all the Ethidium Bromide has been removed. Repeat decontamination procedure as necessary.
* Place all contaminated towels, pads and other debris in a secondary container (non-leaking) with a lid. Label as "Hazardous Waste, Ethidium Bromide (Mutagen)".
* Contact Laboratory Safety Manager at 7-8698.
* After hours contact 7-8763 (Campus Safety) or Emergencies 911

**Note:** Full protective equipment (as described in “Personal Protection” above) should be worn when cleaning up spills of stock solution, powder or crystals.

**Emergency Exposure Procedures:**

***Note****:* ***all spills involving human skin, eye or mouth contact must be reported and an incident report filed with laboratory safety manager and PI. The victim should immediately seek medical evaluation at Claremont Student Health Service, if the exposure occurs in after hours, go to urgent care.***

* **Eye:** If EtBr comes in contact with the eyes, immediately flush them with copious amounts of cold or cool water for a minimum of 15 minutes, preferably in an emergency eye wash.
* **Skin:** In the event of skin exposure, remove contaminated clothing and immediately wash the affected area with copious amounts of cold or cool water for a minimum of 15 minutes.
* **If swallowed or inhaled**: In the case of EtBr ingestion, obtain medical attention immediately. If EtBr is inhaled move the victim to a source of fresh air.
1. Waste Disposal
* Ethidium Bromide **Stock Solutions** and **Powder**- or **Crystal**-contaminated materials must be managed and disposed of ***as a hazardous waste.***
* Agarose gels with trace amounts of Ethidium Bromide (0.3 - 0.5 μg/ml) are to be dried (in fume hood) and then packaged into bags for disposal by EH&S.
* **Buffer Solutions** with trace amounts of Ethidium Bromide must be added to the large container in the fume hood which contains charcoal absorber, stir overnight and recheck for EtBr using hand held UV.
* **Pipette tips** can be disposed of in Ethidium Bromide waste bin and will be removed as per dried gels. **NOTE: Pipette tip collection bin must be closed when not in use.**
* All Ethidium Bromide contaminated waste are to be picked up quarterly by North State Environment and disposed of as a hazardous waste.
1. Decontamination
* Put on heavy duty gloves (preferred, since abrasive pad may puncture thin nitrile gloves) or double layers of nitrile gloves.
* Absorb the spill up with paper towels or some other absorbent and place absorbents in a secondary container that does not leak.
* Once the area is dry, spray the affected area with decontamination solution (10% bleach solution or 1% sodium hypochlorite solution) and allow it to sit for at least 10 minutes.
* Using paper towels dry up the area and then wipe the area down with absorbents dipped in tap water. Repeat this process until the area is clean.
* Using a UV light, check the area to ensure that all the Ethidium Bromide has been removed. Repeat decontamination procedure as necessary.
* Place all contaminated towels, pads and other debris in a secondary container (non-leaking) with a lid. Label as "Hazardous Waste, Ethidium Bromide (Mutagen)".
1. Summary
* If I have any questions I must ask a trained person ***before*** working with Ethidium Bromide and making gels.
* I understand that Ethidium Bromide is a hazardous substance and that I must work in a manner that puts my own safety first.
* I understand that personal safety equipment must be worn at all times and that all work must be done in the fumehood – no exceptions.
* I must also ensure the safety of my colleagues – bottles of EtBr and EtBr contaminated material will not be placed or used in a way that might make contact with other lab personnel.
* I have read and understood the above.

Training Documentation

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