**Standard Operating Procedure**

**DNA Gel Electrophoresis**

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**Purpose**

This SOP covers the procedure for DNA gel electrophoresis (Agarose gels).

**Table of Hazard Properties of Materials Used in This SOP:**

<table>
<thead>
<tr>
<th>Chemical Name</th>
<th>CAS #</th>
<th>Health Hazards</th>
<th>Physical Hazards</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethidium bromide</td>
<td>1239-45-8</td>
<td>- Acutely toxic</td>
<td>- Not flammable or reactive</td>
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<td></td>
<td></td>
<td>- Reproductive hazard</td>
<td></td>
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<tr>
<td>Agarose</td>
<td>9012-36-6</td>
<td>- Inhalation hazard</td>
<td>- Not flammable</td>
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<tr>
<td></td>
<td></td>
<td>- Skin and eye irritant</td>
<td></td>
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<tr>
<td>Tris Base</td>
<td>77-86-1</td>
<td>- Not hazardous</td>
<td>- Not reactive</td>
</tr>
<tr>
<td>Glacial Acetic Acid</td>
<td>64-19-7</td>
<td>- Irritant</td>
<td>- Highly Flammable</td>
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<td></td>
<td></td>
<td>- Corrosive</td>
<td></td>
</tr>
<tr>
<td>EDTA</td>
<td>60-00-4</td>
<td>- Skin/eye irritant</td>
<td>- Not Flammable or reactive</td>
</tr>
<tr>
<td>Sodium hydroxide</td>
<td>1310-73-2</td>
<td>- Corrosive</td>
<td>- Not flammable or reactive</td>
</tr>
</tbody>
</table>

**Summary of Significant Health and Physical Hazards**
- Ethidium bromide is a reproductive toxin and acutely toxic material.

**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.

For non-emergency situations, if an air purifying respirator is used it must be a full-facepiece style mask with high-efficiency filters (HEPA) except for the part of the process when nitric acid is involved where an air purifying respirator cannot be used. There is no commercially available air purifying respirator cartridge rated for protection against nitric acid and the off-gassing products that are oxides of nitrogen, NOx.

A full-facepiece style respirator is required to protect the eyes from contact with the aerosolized corrosive materials.

Lab personnel intending to use/wear a respirator mask must be trained and fit-tested by EH&S. This is a regulatory requirement.

**Hand Protection**

Chemical protective gloves must be worn when handling this material. Nitrile, butyl rubber, or viton/butyl rubber gloves are the materials of choice. Gloves are worn for splash protection only and not for extended contact with these materials. No latex gloves are allowed.

**NOTE:** Consult with your preferred glove manufacturer to ensure that the gloves you plan on using are compatible with the chemicals listed above.

Refer to glove selection chart from the links below:
OR
http://www.allsaftyproducts.biz/page/74172
OR
http://www.showabestglove.com/site/default.aspx
OR
http://www.mapaglove.com/

**Eye Protection**

Wear chemical faceshield over chemical splash goggles or safety glasses with side shields.

**Skin and Body Protection**
- Flame resistant lab coats must be worn and be appropriately sized for the individual and buttoned to their full length as isopropyl alcohol and ethanol are flammable solvents. Laboratory coat sleeves must be of sufficient length to prevent skin exposure while wearing gloves. Personnel should also wear full length pants, or equivalent, and close-toed shoes. Full length pants and close-toed shoes must be worn at all times by all individuals that are occupying the laboratory area. The area of skin between the shoe and ankle should not be exposed.

- Some of the materials in this SOP are corrosive to skin, eyes and nasal tissue – protect these areas from splashes.

Hygiene Measures

- Avoid any contact with these materials. Wash hands after working with the substance.

- Wash thoroughly and immediately after handling. Ethidium bromide is reproductive toxin and acutely toxic and this material must be strictly controlled to prevent exposures.

- Remove contaminated clothing in accordance with approved procedures and dispose of waste in specially designated containers.

Engineering Controls

This SOP should be conducted in a dedicated location with dedicated spill containment provisions.

First Aid Procedures

If inhaled

Evacuate the victim to a safe area as soon as possible. Loosen tight clothing such as a collar, tie, belt or waistband. If breathing is difficult, administer oxygen. If the victim is not breathing, perform mouth-to-mouth resuscitation. WARNING: It may be hazardous to the person providing aid to give mouth-to-mouth resuscitation when the inhaled material is toxic, infectious or corrosive. Seek immediate medical attention.

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 immediately

In case of eye contact

Check for and remove any contact lenses. In case of contact, immediately flush eyes with plenty of water for at least 15 minutes. Cold water may be used. Get medical attention.

If swallowed: Do not induce vomiting. Get medical aid.

Special Handling and Storage Requirements
Ethidium bromide and sodium hydroxide are considered ‘Particularly Hazardous Substances’ and their use must be in a dedicated area using dedicated handling and waste handling procedures.

Handling: Do not get in eyes, on skin, or on clothing. Do not ingest or inhale. Use only in a specially designated area.

Storage: Store all chemicals according to manufacturer’s instructions

Spill and Accident Procedure

Chemical Spill Dial 9-911 and 228-7864

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 9-911 and EH&S at 228-7864 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 228-7864 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 228-7864 immediately.

Medical Emergency Dial 9-911 or 228-7864

Life Threatening Emergency, After Hours, Weekends and Holidays – Dial 9-911 Note: All serious injuries must be reported to EH&S at 228-7864 within 8 hours.

Non-Life Threatening Emergency – Go to the Olivewood Meadows Occupational Health 374 Olive during regular business hours. All other times report to Mercy Medical Center 315 Mercy Ave. Note: All serious injuries must be reported to EH&S at 228-7864 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. Go to the Olivewood Meadows Occupational Health 374 Olive during regular business hours. All other times report to Mercy Medical Center 315 Mercy Ave. Note: All needle stick/puncture exposures must be reported to EH&S at 228-7864 within 8 hours.

Decontamination/Waste Disposal Procedure
- Using proper personal protective equipment as outlined above, decontaminate equipment and bench tops using soap and water and properly dispose of all contaminated disposables as hazardous waste following the guidelines below.

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/](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
- 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 228-7864 for questions
- Empty Containers
  - Dispose as hazardous waste if it once held extremely hazardous waste (irrespective of the container size) A list can be found at [http://ehs.ucla.edu/Pub/ExtremelyHazardousWaste.pdf](http://ehs.ucla.edu/Pub/ExtremelyHazardousWaste.pdf)

Prepare for transport to pick-up location

- Check on-line waste tag
- Use secondary containment

**Safety Data Sheet (SDS) Location**

Risk Assessment

The overall health and safety risk for use of this material in accordance with the procedure and protocol in the following section is considered **LOW** based on:

- The total quantity of any potentially hazardous material handled/transfered any one time is small – milliliter quantities or less.

- Personnel wear protective clothing to prevent skin contact from splashes and for proper clean up practices.

- The primary hazard is a spill or splash from improper or poor handling practices.
Preparatory Steps:

- Review MSDS; special handling, decontamination, and waste disposal information in this SOP; and the emergency information contained in this SOP.

- Visually verify that access to the emergency eyewash/shower unit and the fire extinguisher are not blocked.

- Visually verify that access to the emergency exit door is free obstructions.

- Don the appropriate protective equipment.

- Check equipment and wiring before use. Look for signs of damage. Do not use worn or frayed leads.

- Use only electrophoresis tanks which have a secure design preventing contact with buffer when connected to a power supply.

- Always disconnect from the power supply before opening.

- Switch off power before moving a tank.

- Clean up spills of electrophoresis buffer or gel mixes immediately – these may contain toxic chemicals e.g. ethidium bromide or acrylamide.

Laboratory Procedure

Preparing the 1% Agarose Gel

1. Review MSDS (Material Safety Data Sheets) again – all sections; pay special attention to ACCIDENTAL RELEASE MEASURES, HANDLING AND STORAGE, EXPOSURE CONTROLS/PERSONAL PROTECTION
2. Don Thermal Protection gloves and wear protective goggles; CHECK FOR WEAR AND TEAR
3. Weigh 1.0 g of agarose powder on laboratory scale in clean weighing boat.
4. Transfer the agarose powder to a 250 mL Erlenmeyer flask, add 100 mL of 1X TAE buffer to the flask.
5. Swirl slowly to distribute the agarose powder in the liquid and microwave for 30 seconds. WARNING: Never point the flask opening at you as splashing of hot liquid/vapor may occur while swirling.
6. Check for agarose particle dissolving by swirling slowly and microwave for additional 30 seconds until all the agarose particles are dissolved and solution looks clear.
7. Assemble the gel tray and the comb of appropriate size in fume hood.
8. After the gel cools down to the temperature okay to touch by hand, add 1 μL of Ethidium bromide solution per 10 mL of the gel, and swirl slowly.
9. Pour gently the gel into the gel tray and let it cool down for 25-30 minutes.
10. Remove the comb and store the gel at 4 ºC in a securely sealed container.

Running DNA gel (1% DNA agarose)

1. Don protective gloves (NITRILE) and wear protective goggles; CHECK FOR WEAR AND TEAR.
2. Make sure the electrophoresis apparatus is powered off.
3. Submerge the gel in 1X TAE buffer in buffer tank.
4. Load 8-10 µL of DNA samples and DNA size marker in separate wells.
5. Close the lid of the buffer tank by connecting the appropriate electrodes.
6. Connect the electrical wires to the power supply and run the gel at 100V for 20-30 mins for <2% DNA gel.
7. Power off the electrophoresis apparatus.
8. In case of spill, clean and dispose according to MSDS, and tidy up the working area.

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 the chemicals listed above, 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, requirements, and responsibilities of this SOP:

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