Purpose

This SOP covers the procedure for Protein gel electrophoresis (SDS-PAGE).

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>
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</thead>
<tbody>
<tr>
<td>Acrylamide</td>
<td>79-06-1</td>
<td>- Skin/eye irritant</td>
<td>Reactive with oxidizing agents, acids, alkalis</td>
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<tr>
<td>Bisacrylamide</td>
<td>110-26-9</td>
<td>- Skin/eye irritant</td>
<td>Reactive with oxidizing agents, acids, alkalis</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>Glycine</td>
<td>56-40-6</td>
<td>- Skin and eye irritant</td>
<td>- Reactive with oxidizing agents</td>
</tr>
<tr>
<td>TEMED - tetramethylethylenediamine</td>
<td>110-18-9</td>
<td>- Corrosive</td>
<td>- Highly flammable</td>
</tr>
<tr>
<td>Glacial Acetic Acid</td>
<td>64-19-7</td>
<td>- Irritant</td>
<td>- Highly Flammable</td>
</tr>
<tr>
<td>Coomassie brilliant blue R-250</td>
<td>6104-59-2</td>
<td>- Skin/eye irritant</td>
<td>- Reactive with oxidizing agents</td>
</tr>
</tbody>
</table>

SOP for SDS-PAGE (Protein gel electrophoresis) 1 Date: 07/01/2014
**Environmental Health and Safety**

**SOP for SDS**

**PAGE (Protein gel electrophoresis)**

**SDS – sodium dodecyl sulfate**

- Skin/eye irritant
- Respiratory irritant on inhalation
- Reactive with strong oxidizing agents

**Methanol**

- Skin/eye irritant
- Toxic if ingested
- Highly Flammable
- Explosive in the vapor form

**Ammonium persulfate**

- Skin/eye irritant/sensitizer
- Not reactive

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**Summary of Significant Health and Physical Hazards**

- TEMED: highly flammable liquid and vapour.

- TEMED: causes severe skin burns and eye damage.

- Methanol: highly flammable liquid and vapour.

- Acrylamide and bisacrylamide are toxic chemicals and pose health hazards as potential carcinogen and reproductive toxins.

- Most other buffering agents and chemicals used in this procedure are irritant and corrosive to skin and eye. Wear protective equipments such as eye glasses and gloves while handling these chemicals.

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**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.allsafetyproducts.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.

Handling and Storage Requirements

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. For methanol in particular, keep away from heat, hot surfaces, sparks, open flames and other ignition sources.

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 reports go 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 reports go 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/ 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

Prepare for transport to pick-up location
  - Check on-line waste tag
  - Use secondary containment

Safety Data Sheet (SDS) Location

Online SDS can be accessed at http:// ehs.ucmerced.edu/material-safety-data-sheets.
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/ transferred 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.
Protocol/Procedure

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

Making the SDS-PAGE 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 protective gloves (NITRILE) and wear protective goggles
3. Set up the gel plates.
4. Mix all the components of resolving (running) gel EXCEPT FOR TEMED and 10% APS.
5. Filter and degas the resolving gel solution. Add TEMED and APS right before pouring the gel.
6. Mix the TEMED and APS, and fill the gel plates to about 3/4th height from the bottom.
7. Layer the resolving gel with n-butanol to prevent interference of atmospheric oxygen in acrylamide polymerization.
8. Let the gel polymerize for 30-40 minutes.
9. Remove the n-butanol by pouring down into a sink, and then rinse with distilled water.
10. Dry the excess water by inserting small pieces of filter paper between the plates.
11. Mix the contents of stacking gel similarly as described for resolving gel.
12. Add TEMED and APS to stacking gel solution, mix and pour the solution between the glass plates.
13. Insert slowly the comb between two glass plates to avoid splash, and let the gels polymerize for about 30 minutes.
14. Store the gels at 4ºC in a securely sealed container.

Running the SDS-PAGE gel (17% gel).
1. Don protective gloves (NITRILE) and wear protective goggles; CHECK FOR WEAR AND TEAR.
2. Make sure the electrophoresis apparatus is powered off.
3. Assemble the 2 sandwiched gels in gel running apparatus. Use buffer dam if running only one gel.
4. Remove the comb from the gels.
5. Place the assembly inside the buffer tank and fill the tank with 1X glycine buffer to 3/4th height from the bottom. Pour the buffer between two plates to completely fill the space between the plates.
6. Check the gel plate assembly for leakage of buffer from compartment between two gel plates.
7. Premix 20µL of protein sample with 20µL of 2X SDS loading buffer in microcentrifuge tubes.
8. Boil the sample for 5 minutes at 95ºC and spin down for 1 minute at 13,000 rpm.
9. Load ~6µL of protein marker and ~10µL of protein samples in individual wells.
10. Cover the buffer tank with the lid by connecting appropriate electrode points.
11. Connect the wires to power supply.
12. Run the electrophoresis in 2 steps: Step 1 – 30 minutes at 60V, Step 2 – 1 hour 30 minutes at 140V.
13. Power off the electrophoresis apparatus.

Staining and Destaining the Gel

1. Don protective gloves (NITRILE) and wear protective goggles; CHECK FOR WEAR AND TEAR.
2. After the gel electrophoresis is complete, carefully separate the two glass plates and transfer the gel to a flat plastic container.
3. Rinse the gel with doubly distilled water. Add the Coomassie brilliant blue R-250 staining solution to submerge the gel completely.
4. Cover the container with the lid and incubate the gel with slow shaking for about 1 hour in a fume hood.
5. Drain the staining solution from the plastic box and rinse the gel with doubly distilled water.
6. Pour the destaining solution to submerge the gel and incubate for 30 minutes with slow shaking.

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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<th>Name</th>
<th>Signature</th>
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