 <b>NUS</b> National University of Singapore	<b>Department of Medicine</b>		Doc. No:	SOP-Medicine-16
	<b>Standard Operating Procedure</b>		Rev No:	002
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## 1. OBJECTIVE

The purpose of this document is to outline the safe working procedures for Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) and western blotting in order to protect laboratory personnel and students from potential risks of infections and other risks in the NUS Department of Medicine research laboratories.

*\*The purpose of this SOP template is to outline the general procedures for performing western blot. It is the responsibility of the PI/users to customize the information to match his/her specific operations.*

## 2. SCOPE

This SOP is applicable to all laboratory personnel and students who are conducting SDS-PAGE and western blot in the NUS Department of Medicine research laboratories.

## 3. RESPONSIBILITIES


- 3.1 Principal Investigator in conjunction with the safety lead/supervisor are responsible for making sure that staff are properly trained and equipment and facility are maintained in good working order.
- 3.2 All staff and students must be aware of the potential risks associated with SDS-PAGE and western blotting; must obtain the necessary training and work under supervision until proficient in the practices and techniques to work safely and independently.

## 4. POTENTIAL HAZARDS

- 4.1 Key solutions and reagents used for the running of SDS-PAGE and western blot including polyacrylamide gel, SDS, ammonium persulfate (APS), tetramethylethylenediamine (TEMED), bromophenol blue,  $\beta$ -mercaptoethanol, methanol. It is important that users read the SDS carefully and understand the hazards and risks associated with each chemical before starting work.
- 4.2 All live specimens such as cells, tissues or body fluids from human are potential sources of infection. Potential laboratory hazards associated with human samples/cells include blood borne pathogens e.g. Hepatitis B virus, HIV, etc. Users need to know the risks associated with the biohazards before starting work.

## 5. TRAINING REQUIRMENTS

Complete the relevant safety training [Chemical Safety](#), [Biosafety for BSL-2 Laboratories](#) and [Safe Handling of Human Tissue and Fluids](#) via NUS LumiNUS @ <https://luminus.nus.edu.sg/>

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## 6. IMMUNIZATION

Immunization for hepatitis B is required for all personnel handling human samples.

## 7. PERSONAL PROTECTIVE EQUIPMENT


All personnel are required to wear the following personal protective equipment: long sleeved lab coat, nitrile gloves (or glove material impermeable and resistant to the substance), safety glasses and covered shoes at all times.

## 8. SAFETY PRECAUTION


- Read and understand this SOP and the risk assessment for SDS-PAGE and western blot, along with the safety data sheet (SDS) of chemicals used.
- Do not eat, drink, smoke, handle contact lenses, apply cosmetics, or store food for human consumption in the laboratory. Oral pipetting of any substance is prohibited.
- Wash hands after gloves have been removed, before leaving the laboratory and at any time after handling materials known or suspected to be contaminated.
- Decontaminate all work surfaces before and after the experiments, and immediately after any spill or splash of potentially infectious material with an appropriate disinfectant. Clean laboratory equipment routinely, even if it is not contaminated.
- Know the location of spill kits, eyewashes, safety showers, fire extinguishers before starting work.
- Protein extraction from cells or tissue should be performed in Biosafety level 2 (BSL 2) biological safety cabinet. Know the details and potential risks of the specific samples you are working on before you start handling them.
- Limit preparation of acrylamide work to designated areas where possible. Coat work bench with absorbent bench paper prior to working with acrylamide to prevent contamination of non-disposable surfaces. Change the absorbent bench paper layer frequently or when soiled. For detailed procedure for making and running SDS-PAGE gels, read the instruction manual thoroughly before using the apparatus.
- Ensure gel electrophoresis tank and power supply are in good working order, and that lid is always on electrophoresis tank when in operation. Electrophoresis chamber has a secure LID which prevents accidental contact with the electrified buffer solution.

## 9. PROCEDURE

- Sample preparation (Protein Extraction) from whole cell lysate, cell culture or tissue. Prepared samples need to be denatured. Briefly heat samples in a heat block and centrifuge before storing on ice. Remove molecular marker from freezer and store on ice.

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- Prepare fresh SDS-PAGE gel or remove pre-cast gel from fridge. Unwrap and remove the comb. Set up apparatus according to the manufacturer's instructions.
- Load the samples and molecular maker. Connect the electrodes and run the gel at desired running conditions. If there is any leakage in the system, the top fluid layer will run to the bottom layer. If this occurs, immediately transfer some of the buffer from the bottom chamber to the top chamber. Ensure the electrodes are still immersed in the buffer.
- When the gel run is almost complete, prepare the transfer materials. Cut a piece of blotting membrane to the gel size and soak in transfer buffer. Remember not to touch the membrane with fingers. Always use forceps. Pre-wet a set of filter papers and sponges with transfer buffer.
- Set up the transfer tank according to the manufacturer's instructions. Pour cold transfer buffer into the tank just above the minimum level.
- When the gel has finished running, carefully remove the gel and soak in transfer buffer. Wash for a few minutes to remove any residual SDS.
- Using forceps, place the pre-wet membrane on top of the gel. Place damp filter paper onto the membrane. Turn over the sandwiched membrane so the filter paper lies underneath. Use a second piece of damp filter paper on the gel back. As protein will not transfer where there are air bubbles, ensure any air bubbles between gel and filter paper are gently rolled out.
- Dampen the sponges and place the western blot transfer sandwich into the transfer cassette. The order of the western blot transfer sandwich should be: negative side, sponge, filter paper, gel, membrane, filter paper, sponge, positive side.
- Close the plastic cassette case and place in the tank with the clasp towards the top. To reduce the heat, place ice packs inside the gel tank or set up the apparatus in cold room. Connect the electrodes and run the transfer sandwich at desired transferring conditions.
- Once transfer is complete, wash the membrane in appropriate washing buffer and block the membrane in appropriate blocking buffer with shaking.
- After blocking, wash the membrane and incubate the membrane with primary antibody specific to target protein with shaking. Wash the membrane.
- Incubate the membrane with specific enzyme-conjugated secondary antibody with shaking. Wash the membrane.
- Proceed with chromogenic, chemiluminescence, or fluorescence immunodetection to develop the membrane until appropriate bands appear.

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## 10. WASTE DISPOSAL

### 10.1 Cytotoxic Waste:

- Wastes generated from or which have been in contact with polyacrylamide gel (tubes, pipette tips, gloves, paper towels) with addition of APS, TEMED and acrylamide are treated as cytotoxic wastes and must be disposed in double cytotoxic purple bag. Collect the liquid waste in carboy container with 'Cytotoxic Waste' labeled. The carboy is placed in a secondary container. Both liquid and solid wastes are to be collected by the licensed waste contractor.

### 10.2 Chemical Waste

- The experiment produces hazardous chemical liquid wastes such as the running and transfer buffer. Chemical waste should be collected in a compatible waste container. NUS Hazardous Waste Label and GHS label must be placed on the container upon the start of accumulation. Wastes are collected by the licensed waste contractor. It is good practice to dispose chemical waste within 90 days from date of generation.

### 10.3 Biohazard Solid Waste

- Solid biohazard waste should be double bagged (yellow bag) in the disposal bin with biohazard label. When the bag is 2/3 full, tie both biohazard waste bags tightly. Label bag with lab location, PI name and contact number. The biohazard waste will be collected by the licensed service provider.

### 10.4 Biohazard Liquid Waste

- Wash waste generated from the assays should be disposed of as biohazard waste.
- Liquid waste is treated with appropriate disinfectant e.g. Presept before flushing down the sink with plenty of water.

## 11. SPILL RESPONSE


If spillage occurs, inform the PI/supervisor/safety lead and spill responders immediately and refer to Spill Clean-Up Procedure: SOP-Medicine-01 Biological Spill Response and/or SOP-Medicine-03 Chemical Spill Disposal.

## 12. INCIDENT REPORTING

Accidents resulting in injuries must be reported to the PI and/or laboratory safety lead immediately after first aid is applied.

Seek medical attention when necessary at the University Health Centre or proceed to the Accident & Emergency units of National University Hospital after office hours.

All incidents or accidents have to be notified to OSHE within 24 hours via the online NUS Accident and Incident Management System (AIMS)

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@[https://inetapps.nus.edu.sg/osh/portal/eServices/ehs360\\_aims.html](https://inetapps.nus.edu.sg/osh/portal/eServices/ehs360_aims.html). The AIMS report can be submitted by the injured staff/student, safety leads, his or her supervisor/representative if the staff or student is unfit/unable to do the initial report.

### 13. REFERENCES

- a. NUS Laboratory Biorisk Management Manual (OSHE NUS/OSHE/M/01)
- b. NUS Laboratory Chemical Safety Manual (OSHE NUS/OSHE/M/02)
- c. SOP-Medicine-01 Biological Spill Response
- d. SOP-Medicine-02 Biological Waste Disposal
- e. SOP-Medicine-03 Chemical Spill Disposal
- f. SOP-Medicine-04 Chemical Waste Disposal

### 14. REVISION HISTORY

Date Revised	Version No.	Author	Summary of Revisions
29-09-2020	002	Seet Bee Leng	Update of (5) Training Requirements- NUS online training portal and (7) Personal Protective Equipment.