## 1. OBJECTIVE

The purpose of this document is to outline general procedures for safe handling of DNA extraction using phenol-chloroform and isoamyl alcohol in order to prevent and minimize laboratory personnel from exposure of toxic vapours and mists, as well as chemical contaminated materials.

**The purpose of this SOP template is to outline the general procedures for performing DNA extraction by phenol-chloroform and isoamyl alcohol. 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 DNA extraction using phenol-chloroform and isoamyl alcohol in the NUS Department of Medicine research laboratories.

## 3. RESPONSIBILITY AND ACCOUNTABILITY

3.1 It is the responsibility of the Principal Investigators and/or in conjunction with the laboratory supervisor/safety lead to ensure all users receive proper training and necessary medical examination before commencing work. They are also responsible in communicating the chemical hazards involved to users and their staff and students understand the safe use and handling of chemicals, as well as correct operation and maintenance of equipment.

3.2 All staff and students are required to acquire adequate knowledge of the chemicals and the potential risks associated with hazardous chemicals that they are handling. They must attend the necessary training and work under supervision until proficient in the practices and techniques to work safely.

## 4. SAFETY TRAINING

All personnel (staff and students) handling hazardous chemicals and biohazard materials are required to complete the relevant safety training Chemical Safety and Biosafety for BSL-2 Laboratories. Personnel working with body fluids and tissues from humans in laboratory setting are required to complete the Safe Handling of Human Tissue and Fluids vial IVLE @ https://ivle.nus.edu.sg/.

## 5. IMMUNIZATION

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

## 6. PERSONAL PROTECTIVE EQUIPMENT

All workers shall wear long sleeved lab coat, covered shoes, safety glasses/goggles and nitrile gloves (or glove material impermeable and resistant to the substance).
Wear appropriate face shield and insulated gloves if necessary.

## 7. ENGINEERING CONTROL

All mixing and preparation with phenol and chloroform must be performed in a properly operating and certified chemical fume hood.

When handling biological samples, work must be done in a biosafety cabinet level 2, BSL2, to avoid any infectious splashes or aerosols generated during microbiological procedures.

Emergency eye wash and safety showers must be available in the immediate vicinity of use or handling.

## 8. HAZARDS ASSOCIATED WITH DNA EXTRACTION

8.1 Biological Hazards:
Potential laboratory hazards associated with human materials include bloodborne pathogens e.g. Hepatitis B virus, HIV, etc. and all staff and students should be aware of the risks associated with the biohazards before starting work.

8.2 Chemical Hazards:
Phenol is very hazardous in case of skin or eye contact, ingestion or inhalation.
Chloroform is hazardous in case of skin contact, eye contact, or if ingested or inhaled.
Isoamyl alcohol and ethanol is flammable and irritating if it gets in the eye.
Refer to the chemical supplier's safety data sheet (SDS) before using these chemicals and disposing of spent chemicals and chemical wastes.

8.3 Equipment Hazards:
Pipetting and centrifugation may result in the formation of aerosols and droplets. Refer to manufacturer's instruction manual for safe use of pipettes centrifuge.

## 9. SAFETY PRECAUTIONS

- Know where the nearest emergency eyewash station and safety shower are in the laboratory before beginning work. Both equipment are routinely checked.
- Ensure that a spill kit is well stocked and readily available should a spill occur.
- Make sure you are using appropriate tubes for the procedure – polypropylene is best, and the cap must be very tight-fitting. Do not use polycarbonate tubes – these will be dissolved by the phenol-chloroform. Glass is not recommended due to risk of breakage.
- Wash hands thoroughly with soap and water after work is completed
- Refer to the SOPs on the safe use of equipment: magnetic stirring plate, autoclave, biosafety cabinet, fume hood, centrifuges, water bath, etc.
- Refer to the SDS prior to the use of chemicals.

## 10. PROCEDURE

10.1 Cell Lysis and Digestion (this procedure prior to DNA extraction differs from laboratory to laboratory. Please refer to in-house protocol.)

10.1.1 DNA Extraction from Blood:

- Transfer blood in EDTA to appropriate sterile tube.
- Centrifuge at appropriate speed at 4$^{o}$C.
- Carefully remove the supernatant (plasma).
- Fill tube with lysis buffer. Shake well and rotate to lyse the red blood cells.
- Centrifuge at appropriate speed at 4$^{o}$C. Gently discard supernatant.
- It may be necessary to repeat above step to get a cleaner/purer pellet.
- Add 10% SDS and proteinase K to the viscous mixture.
- Incubate tube while rotating at 37$^{o}$C for at least 6 hours or overnight or at 55$^{o}$C for at least 4-5 hours until sample is clear with no visible lumps. Otherwise, gently invert the tube a few times during incubation. Proceed to phenol-chloroform extraction.

10.1.2 DNA Extraction from Cultured cell Lines:

- Trypsinize cells if in a monolayer.
- Pellet cells by centrifugation at appropriate speed at 4$^{o}$C.
- Remove culture medium and wash cells with PBS
- Resuspend cells in lysis buffer and mix well by gentle pipetting.
- Centrifuge at appropriate speed at 4$^{o}$C. Gently discard supernatant.
- Add 10% SDS and proteinase K to the viscous mixture.
- Incubate tube while rotating at 37$^{o}$C for at least 6 hours or at 55$^{o}$C for at least 4-5 hours until sample is clear with no visible lumps. Otherwise, gently invert the tube a few times during incubation. Proceed to phenol-chloroform extraction.

10.1.3 DNA Extraction from Tissues:

- Mince fresh, solid tissue into smaller pieces with a sterile scalpel blade or grind the tissue (with the pestle) filled in a clean mortar with liquid nitrogen.
- Place tissue sample into appropriate sterile tube.
- Add lysis buffer to tube. Ensure that the tissue is completely immersed in the lysis buffer.
- Mix by gentle pipetting.
- Add 10% SDS and appropriate amount of proteinase K to the viscous mixture.
- Incubate tube while rotating at 37$^{o}$C for at least 6 hours or at 55$^{o}$C for at least 4-5 hours until sample is clear with no visible lumps. Otherwise, gently invert the tube a few times during incubation. Proceed to phenol-chloroform extraction.

10.2 Phenol-chloroform Extraction of DNA

- Add an equal volume of equilibrated phenol into the above cell solution. Close the cap tightly and mix .
- Centrifuge at appropriate speed at room temperature.

- Transfer top aqueous layer into a new tube taking care not to carry over phenol or white proteinaceous material from the interphase. Discard bottom layer. Repeat the phenol extraction steps if necessary.
- Add an equal volume of either chloroform or chloroform-isoamyl alcohol 24:1. Vortex the mixture.
- Centrifuge at appropriate speed at room temperature.
- Transfer top aqueous layer into a new tube. Discard bottom layer. Repeat the chloroform extraction steps if necessary. This helps to eliminate all the phenol from the DNA sample.

10.3 Purification **and Precipitation of DNA**

- To the aqueous DNA solution add 0.1 volume of 3M sodium acetate (pH 5.2) and mix well by votexing.
- Add 2 volumes of ice cold 100% ethanol to the tube. Close the cap tightly and mix by inversion. A white precipitate should form.
- Centrifuge at appropriate speed at room temperature.
- Gently aspirate out the supernatant and discard it.
- Add cold 70% ethanol to the precipitated DNA.
- Centrifuge at appropriate speed at room temperature.
- Gently aspirate out the supernatant and discard it.
- Allow the precipitate to air-dry DNA until the white precipitate becomes clear.
- Dissolve the precipitate in an appropriate volume of TE buffer. Failure to adequately resolubilize the DNA will result in uneven distribution of DNA within the solution.
- Store the purified DNA sample at 4$^{o}$C prior to analysis or indefinitely at -20$^{o}$C.

## 11. DISPOSAL OF WASTE

11.1 Biohazard Solid Waste

- Solid biohazard waste such as culture flasks, centrifuge tubes, contaminated gloves, tissues, etc. should be double bagged (yellow bag) in the disposal bin with biohazard GHS label. When the bag is 2/3 full, tie both biohazard waste bags tightly. Label bag with PI name and contact number. The biohazard waste will be collected by the licensed service provider.

11.2 Biohazard Liquid Waste

- Biohazard liquid waste including must be treated with appropriate disinfectant - either inactivate for at least 1 hour in freshly prepared Presept solution (5,000 ppm) or appropriate disinfectant prior to disposal to drain with an excess of water.

11.3 Chemical Liquid Waste

- Chemical liquid waste shall be collected in compatible chemical waste container (Refer to EPA's Chemical Compatibility Chart for determining the chemical compatibility of the waste with the waste container). Incompatible chemical liquid wastes shall not be mixed and stored together. 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.

## 12. 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.

## 13. 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) @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.

## 14. REFERENCES

a. NUS Laboratory Chemical Safety Manual (OSHE NUS/OSHE/M/02)
b. NUS Laboratory Biorisk Management Manual (OSHE NUS/OSHE/M/01)
c. SOP-Medicine-04 Chemical Waste Disposal
d. SOP-Medicine-02 Biological Waste Disposal
e. EPA's Chemical Compatibility Chart https://share.nus.edu.sg/corporate/procedures/safety_and_health/Chemical-Safety-Procedures/EPAChemicalCompatibilityChart.pdf