***STANDARD OPERATING PROCEDURE – E008***

**SONICATION**

1. **Objectives**

The objective of this document is to establish standard operating procedures for the process of sonication, ensuring the safety of laboratory personnel by mitigating potential risks associated with hazardous materials, and injuries. Additionally, this SOP aims to enhance the efficiency of experimental workflows.

1. **Personal Protective Equipment**

To ensure safety during sonication, appropriate personal protective equipment (PPE) must be worn. This includes:

* Earmuffs which must fully cover ears; communal units should be disinfected with 80% v/v ethanol before use.
* Long pants and closed-toe shoes to protect against spills and splashes.
* A long-sleeved, buttoned lab coat to minimize skin exposure.
* Safety glasses or goggles to protect against splashes or flying debris.
* Disposable nitrile gloves to prevent direct contact with hazardous chemicals.
* If the user has long hair, it must be tied back.

1. **Potential Hazards**

Sonication presents various hazards that must be managed to maintain a safe working environment. This includes:

* **Hearing Damage:** Prolonged exposure to high-frequency noise without ear protection.
* **Physical Injury:** Probe shattering due to misuse (e.g., touching container walls or dry operation). Burns from overheating equipment or hot samples.
* **Biological Hazards:** Aerosol generation (if sonicating infectious materials; must be performed in a biosafety cabinet for Risk Group 2+ organisms).Cross-contamination between samples.
* **Chemical Hazards:** Splashes or spills of hazardous chemicals (e.g., solvents, toxins).Static electricity buildup when sonicating flammable liquids.
* **El**ectrical Hazards: Faulty wiring or improper grounding leading to shocks. Overheating due to extended use without rest periods.

1. **Procedures**

A. Preparation

* Seek instructions from the equipment custodian before use.
* All chemical SDS should be read and understood prior to starting work. All risks relating to the chemical should be understood.
* Read and understand all information regarding the sonicator before use. If you are unclear about any risks, consult the supervisor or equipment custodian.
* Close all doors that provide access to the room housing the sonicator.
* Ensure everyone in the sonicator room has ear protection. If not, ask them to use ear protection or leave the room.

B: Sonication Probe

1. The probe is used with minor volumes of aqueous suspensions and solutions. It is typically used for bacterial cell disruption and protein release.
2. Determine the approximate volume of the sample that needs sonication.
3. Select the **appropriate sonication probe tip** according to the following guidelines:
   * Sample volume 0.5 mL – 5 mL: Use Double Stepped Microtip
   * Sample volume 5 mL – 20 mL: Use the Tapered Microtip
   * Sample volume 20 mL (Maximum ~100 mL): Use the Step Horn with threaded body.
4. If not already attached, securely screw on the required probe tip to the sonicator unit.
   * **NOTE: For probes to function properly, they should be screwed on tightly**. Avoid frequent unscrewing and screwing to prevent thread deterioration. If necessary, use a spanner and grip unit for these procedures.
5. Unless using a pair for personal use, all communal earmuffs should be wiped with 80% v/v ethanol as a step for decontamination before use.

* **NOTE:** Earmuffs should completely cover the ears for adequate protection.The tube/beaker containing fluid to be sonicated should be placed on ice and inside another container. Set-up the samples and place them on an adjustable platform.

1. Place the probe inside the fluid for sonication and adjust the platform so the end of the probe is half-submerged into the solution. **DO NOT touch the container bottom or side with the probe.**
2. **Close the doors of the Noise Reduction Cupboard.**
3. Turn on the power and set to the required duty cycle.
4. Ensure the Output Control Dial is set so the Output Limit does not exceed the maximum to prevent damage to the sonicator probe tip.
5. While actively using the sonication probe, do not touch the tip on solid surfaces; keep it immersed in liquid without touching the container bottom or sides.
6. Set a timer for the required duration, sonicate for 30 seconds, and then switch it off. The unit should rest for at least a minute to prevent probe overheating. Should overheating occur, allow additional rest time before resuming sonication. A typical program consists of 5 cycles of 30 seconds on and 1 minute off. The sonication probe should be thoroughly cleaned with 80% v/v ethanol in between sonicating different samples.
7. Power off the unit, remove earmuffs, and decontaminate with 80% v/v ethanol.

* Thoroughly clean the sonication probe with 80% v/v ethanol and then wipe dry.

C: Sonicating Waterbath

The sonicating waterbath contains water with a depth of 5 cm and is typically used for glassware cleaning, breaking cell clumps, or cell removal from surfaces. When wearing earmuffs, decontaminate with 80% v/v ethanol. Wear them to fully cover your ears.

Procedures:

1. Ensure the water level of the waterbath should be the same as the sample.
2. Place your sample (e.g., a Falcon tube with a cell or soil suspension) inside a clean glass beaker or similar vessel and fill it with water to match the waterbath level.
3. Put the beaker in the waterbath. Switch on the power (you may need to set the timer to 'hold' as well).
4. Sonicate for a maximum of five minutes at a time. Rest the machine for five minutes or longer between each cycle to prevent overheating.
5. Never allow a microtip or extender to vibrate in air.
6. Turn off the machine, remove earmuffs, and decontaminate with 80% v/v ethanol.
7. **Equipment Repairs** 
   * Should the user encounter any electrical issues prior to use (e.g. failure to turn on equipment, burning smell or sparks occurring during use), discontinue use of the equipment immediately.
   * Any broken equipment should be reported to the supervisor / equipment custodian and equipment taken for repair.
8. **Spills and Incident Reporting**

* In the event of a spill, promptly inform nearby occupants and the departmental safety officer (DSO) and follow the spill response procedures
* If necessary, refer to the corresponding chemical SDS during spill cleanup procedures.
* Any accidents that result in injuries must be reported to the PI and/or the departmental safety officer (DSO) immediately. Near Misses (hazardous situations not resulting in incidents) should also be reported.
* In the case of serious incidents, immediately inform the security unit by calling the 24-hour hotline on 2358 8999.

1. **References**

* Nikolic, A. (2016). *SOP\_SMB030.2: Sonication.* Risk Assessment. The University of Sydney.
* Coleman, N. & Nikolic, A. (2014). *SOP SMB030.2 (AN NC 0614): Sonication.* The University of Sydney*.*