THE UNIVERSITY OF SYDNEY

STANDARD OPERATING PROCEDURE

Procedure	**Use of Polytron tissue homogeniser**
School/Department:	School of Molecular Bioscience
SOP prepared by:	Sue Ling Lim and Markus Hofer
Version:	SMB062.1

Section 1 - Personal Protective Equipment (PPE)

1. Lab coat
2. Gloves
3. Long hair tied back
4. Proper enclosed shoes
5. Ear muffs
6. Safety glasses and fumehood sash drawn down as low as possible (without interfering with experimental work)

Section 2 – Potential Hazards

1. Homogeniser is quite loud, can cause hearing damage if ear muffs are not worn
2. Splash back or spillage of protein lysates or RNA/DNA lysates that contain detergents (e.g. SDS, NP-40) or phenol (e.g. Trisure, TriReagent, Trizol)
3. Workers with pre-existing medical conditions (e.g. allergy, immunocompromised state, chemical sensitivity) and workers who are pregnant or expecting pregnancy must consult with their supervisor AND medical specialist AND the university's WHS services before performing this procedure. If there are any serious concerns expressed by any of these individuals, this task must not be performed.

Section 3 – Procedure

1. Ensure homogeniser probe is cleaned before and after use. If doing RNA work, soak bottom half in 1%(w/v) SDS for 1 hour before use, then rinse thoroughly with milliQ-water. For protein work, pull apart the probe and clean thoroughly with soap and water. Remember to not lose the O-ring when dismantling the larger probe. When inserting probe back into the homogeniser, ensure the top of the probe is dry, and the there is a ‘click’ when inserted. If it has not clicked, it has not been inserted properly and may damage the probe.
2. Wipe down fumehood and line the fumehood with Benchcote before processing samples
3. Aliquot lysis buffer or Trireagent/Trisure/Trizol in 14ml polypropylene tubes or 2ml tubes on ice. Add frozen sample to each tube prior to homogenising. Ensure they do no overflow when the probe is inserted. Homogenise samples on settings / time as required (note: do not homogenize to long to avoid shearing of DNA/RNA). Turn homogenizer off between samples and rinse between samples as necessary. If at any time the homogeniser makes any strange noises or there are any irregularities with use, switch it off immediately via emergency button on fumehood and contact your supervisor. Do not run the homogenizer dry.
4. Upon completion, rinse probe in RO water, then remove it from the homogeniser for cleaning with soap and water.
5. DiscardBenchcote into the biohazard waste and wipe down fumehood and homogeniser with 80%(v/v) ethanol.

Section 4 – Disposal / Spills / Incidents

1. Clean up spills of phenol containing solutions immediately with absorbent material.
2. Dispose phenol containing waste in “phenol waste” container.

Section 5 – Repairs / Certification / Validation

1. If homogeniser makes any unusual sound, stop immediately and contact supervisor.
2. If the homogeniser requires repairs, decontaminate with 80%(v/v) ethanol and submit a jobsheet before taking it down to the workshop.

Section 6 – Relevant safety data sheets (to be available and accessible)

1. Trireagent/Trisure/Trizol, PMSF, NaFl, TritonX, Tween20, NP-40, protease and phosphatase inhibitor cocktails.

Section 7 - References
1. Protein purification and nucleic acid purification using phenol chloroform RAs and SOPs or manufacturer datasheet.

SOP Consultation, Training and Approval

Print names and enter signatures and dates to certify that the persons named in this section have been consulted/trained in relation to the development and implementation of this Standard Operating Procedure. WHS Representative (WHS Committee) certifies that consultation has taken place.

Position	Name	Signature	Date
Supervisor			
employee / student			
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Name Authorising (Printed): *DIANNE FISHER*.....

Signature: .....***Date:*** *14/7/15* .....

WHS Committee Representative Name (Printed): *MARKUS HOFER*.....

Signature: .....***Date:*** *14/7/15* .....