**Standard Operating Procedure**

**Extraction of lipids and acidification of biological material prior to stable isotope analysis**

COSHH Assessment: Extraction of lipids and acidification of samples prior to stable isotope analysis COSSH

Risk Assessments: Extraction of lipids and acidification of samples prior to stable isotope analysis RA

Chemicals Required:

|  |  |  |
| --- | --- | --- |
| Chemical | Hazard Symbols | H-phrases |
| Chloroform | A drawing of a person  Description automatically generatedA drawing of a person  Description automatically generated | H302: Harmful if swallowed  H315: Causes skin irritation  H319: Causes serious eye irritation  **H331**: Toxic if inhaled  H336: May cause drowsiness or dizziness  **H351**: Suspected of causing cancer  **H361**d: Suspected of damaging the unborn child  H372: Causes damage to organs (Liver, Kidneys) through prolonged or repeated exposure  H412: Harmful to aquatic life with long lasting effects |
| Methanol | A picture containing drawing  Description automatically generatedA drawing of a person  Description automatically generatedA drawing of a person  Description automatically generated | H225: Highly flammable liquid and vapour  H301+311+331: Toxic if swallowed, in contact with skin or if inhaled  H370: Causes damage to organs (Eyes) |
| Hydrochloric acid | A close up of a sign  Description automatically generatedA drawing of a face  Description automatically generated | H290: May be corrosive to metals  H314: Causes severe skin burns and eye damage  H335: May cause respiratory irritation |

Other Hazards:

|  |  |  |
| --- | --- | --- |
| Grinder | Logo, company name  Description automatically generated |  |
| Heat plate | A sign on a pole  Description automatically generated |  |
| Biological infection | A drawing of a face  Description automatically generated |  |
| Sonicator/vibrations | A close up of a sign  Description automatically generated |  |



**WARNING**: Chloroform is a CARCINOGEN. Other laboratory users must be made aware.

* All chemicals are to be used in an exhausting fume cupboard and stored in a vented cabinet when not in use.
* Store Chloroform in an acid cabinet. Discard six months after opening (decomposes in contact with air).
* PPE to be worn – Lab coat, nitrile gloves suitable for solvent use, and safety goggles

**Protocol lipid extraction:**

1. Weigh empty glass vial and re-weigh after adding tissue. Record the wet weight of the

sample.

1. Pre‐freeze at ‐80C for 1‐2 hours before lyophilisation.
2. Lyophilise samples for 24‐48h depending on the size of the sample and nature of the tissue.

(Fatty tissues tend to take longer than tissues with high water content).

1. Grind samples using the automatic tissue grinder or mortar and pestle.
2. Separate the sample into approximately two equal portions. Keep one half of the sample in the original glass vial for lipid extraction and transfer the second half of the sample to an Eppendorf vial.

**Steps 6 to 11** must be carried out in an externally exhausting fume cabinet

1. Prepare Chloroform:Methanol mixture (2:1 v/v/v) in a glass stock bottle. Choose the size of the bottle according to the volume of solvent needed to extract all your samples but maximum size 1 litre. If a large amount of samples need to be extracted this process will need to be repeated several times. Volumes of solvents should be determined in glass measuring cylinders.
2. Add solvent mixture (approx. 20x the sample size) to one half of the dried and ground

sample tissue using an appropriate glass measuring pipette.

1. Sonicate samples in a water bath for 30min.
2. Centrifuge at 3400rpm for 10min. Allow centrifuge to rest for 2 minutes before opening lid to prevent aerosol exposure.
3. Pipette off as much of the solvent as possible. Put the solvent waste into a glass bottle clearly labelled with information on the type of solvent contained, hazard labels appropriate to the solvents contained, date and your name.
4. Repeat the extraction process until supernatant appears clear (1‐3 times).
5. Add distilled water to remove traces of solvents.
6. Sonicate samples in a water bath for 30min.
7. Centrifuge at 3400rpm for 10min.
8. Pipette off as much of the water as possible. Transfer the water to the same waste bottle used for the solvents as this water will still contain traces of both chloroform and methanol.
9. Repeat washing process if necessary (1‐2 times).
10. Dry the sample at 50°C in a heating block for at least 24hours.

**Protocol Acidification:**

Samples with high inorganic carbon content need to be acidified

Reagents: 0.5N Hydrochloric acid (In hydrochloric acid Normality = Molarity)

1. Prepare 0.5N HCl in a glass stock bottle. The size of solvent bottle will depend on the volume need for acidification of all samples.

**To make a 0.5N solution of 500ml you need to add 20.5ml of acid to 500ml of water.**

Calculation as follows: For 0.5l of 0.5M HCl solution at 12.2M (molarity of conc. HCl)

Molarity of conc. HCl \* x = Molarity of solution \* Volume of solution

12.2M\*xl = 0.5 M\* 0.5l

X (l)= (0.5M \* 0.5l)/12.2 M

X = 0.0205 L per 0.5 L water

X = 20.5ml HCl per 500ml water

**ALWAYS ADD ACID TO WATER** (NOT to acid)

1. Add acid solution to the lipid‐extracted tissue sample drop by drop until the foaming stops and all inorganic carbon is dissolved.
2. Add vial to centrifuge and spin for 10min. at 3400rpm.

Pipette off as much of the acid as possible. Put the solvent waste into a glass bottle clearly labelled with information on the type of solvent contained, hazard labels appropriate to the solvents contained, date and your name. A new waste bottle needs to be started for this process. Do not mix the acid waste with the previous solvent waste

1. Dry the remaining tissue in a heating block at 50°C until dry (at least 24hours)

**Emergency Procedures**

* First Aid: BAS standard procedures for chemicals.
* Chemical Spill: BAS standard procedures for chemical spill response.
* Fire Extinguisher: water, dry chemical or carbon dioxide

Emergency contact numbers are displayed at laboratory phone.

**Waste Disposal**

* Dispose of any contaminated waste such as gloves into the appropriate bin disposal as biological waste.
* Dispose of waste chemical solutions and chemically contaminated plastics into designated chemical waste containers for disposal as chemical waste.