

**PHYTOLITH REFERENCE SLIDES – CELLS IN SITU**

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**Equipment**

1. Ultrasound bath
2. Furnace
3. Crucibles with lids
4. Glass beakers
5. Fume cupboard
6. 50ml centrifuge tubes
7. 7ml glass vials
8. Small spatula
9. Cocktail sticks
10. Slides
11. Slide covers
12. Precision scales

**Consumables**

1. Sodium hypochlorite (lab grade)
2. Distilled water
3. Entellan

**Personal Protective Equipment**

Lab coat  
Gloves  
Eye protection  
Fume cupboard

**Protocol**

This should all be carried out in the Penn Paleocology Lab, in the fume hood.

1. Cut leaves into small pieces and wash them in distilled water. Place them in a large glass beaker full of distilled water. Place beakers in ultrasound bath for 15mins. Change water and place in the ultrasound bath for a further 15mins.
2. Wash with distilled water and dry in oven. Weigh once dry and place in crucibles.
3. Char plants at 500°C/932°F for 5 hours, until reduced to white ash. Place a ceramic lid over each crucible to prevent material from flying away/contamination!
4. Transfer ash to 50ml centrifuge tube and add sodium hypochlorite (bleach) up to 50ml mark. Leave overnight.
5. Wash bleach away by placing in centrifuge at 2000rpm for 5mins then pour away supernatant.
6. Top-up with distilled water to the 50ml mark and repeat centrifuge and pouring stages until 6 washes (one chemical, five water) completed.
7. Label and weigh 7ml glass vials. Transfer residue from centrifuge tubes and leave to dry in oven at 30-50°C/86-122°F.
8. Clean microscope slides with a little methanol and label then weigh them.
9. Mix the dry sample with a spatula then transfer a few milligrams (around 0.0005g) to slide and weigh slide again. Add a few drops of Entellan to slide and mix using pointed metal object or cocktail stick. Place cover slip over slide.
10. Place in fume cupboard and leave to dry for two weeks.

**Disposal considerations IMPORTANT**

Any supernatant that has hazardous chemicals must be disposed according to University procedure via the appropriate chemical waste bottles and liaison with the lab technician.