

PHYTOLITH REFERENCE SLIDES – CELLS IN SITU

Equipment

1. Ice cube tray
2. Ultrasound bath
3. Aluminium foil
4. 100ml beakers
5. Forceps
6. Pipette
7. Slides
8. Slide covers

Consumables

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

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. Number samples and ice cube trays. Prepare sample by dividing into the parts you want to analyse and make sure they are not folded or bent into funny shapes. If very dusty wash under tap then in distilled water.
2. Place each part into its own beaker and add distilled water. Place in ultrasound bath for 10 minutes.
3. Using forceps transfer plant part to ice cube tray CAREFULLY, trying not to fold the sample or damage it.
4. Fill iced cube trays with sodium hypochlorite (bleach) till half-full (if big sample then a little more can be added – ensure it is submerged). Make sure there are no air bubbles on sample.
5. Cover with foil and leave in fume cupboard. Check after half an hour for air bubbles.
6. Timing depends on size of sample: for leaf/culm etc... 5-7 hours. For inflorescence less than 5 hours. Check regularly to see how well bleached the sample is. Too little time and the tissue remains, too long and slide mounting will be difficult.
7. Remove bleach with pipette VERY CAREFULLY so as not to disturb the sample. Add distilled water.
8. After one hour pipette off water and refresh with new water.
9. Cover with foil and leave overnight in fume cupboard. If clear the next morning, carefully lift sample with forceps and spread on slide, ensuring it does not fold or twist.
10. Place cover slip on straight away and seal in the four corners with nail polish.
11. After a few days the water will have evaporated, you can now seal the entire slide edge with nail polish

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.