

## **Trypan Blue Staining**

### **Materials**

- *2 x Trypan Blue stain.*
  1. 60 ml Lactic acid (Sigma L-1250)
  2. 60g Phenol
  3. 60ml 100% Glycerol
  4. 60 ml dH<sub>2</sub>O
  5. 60mg Trypan blue
  
- *Chloral hydrate de-stain solution.*
  1. 25g Chloral hydrate
  2. 10ml dH<sub>2</sub>O

### **Method**

1. Dilute Trypan Blue stain solution 1:2 with ethanol before use
2. Immerse leaf samples in trypan blue stain solution.
3. Place bottles in a pan of boiling water.
4. Leave bottles in the pan until the stain in the bottle also boils. Let sit for 3 mins.
5. Take the pan off the heat, with the bottles still inside and leave to cool in pot for 1 hours (30 min).
6. Transfer samples to de-stain solution and view the next day.
7. Mount in water or 50% glycerol.

### **Notes**

Leaves can be cleared before Trypan Blue staining to remove chlorophyll and other pigments.