

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 dH₂O
 5. 60mg Trypan blue

- *Chloral hydrate de-stain solution.*
 1. 25g Chloral hydrate
 2. 10ml dH₂O

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 hour (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.