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modified from (O'Brien et al., 1965 & Ciera's protocol)

Toluidine blue in phosphate buffer
Solution A (Monobasic Sodium Phosphate 0.2M)
Monobasic Sodium Phosphate monohydrate ($NaH_2PO_4 \cdot H_2O$) – 2.76g
Distilled Water - 100ml

Solution B (Sodium phophate dibasic 0.2M)
Sodium phosphate dibasic heptahydrate ($Na_2HPO_4 \cdot 7H_2O$) – 5.37g
Distilled Water - 100ml

Phosphate buffer 0.1M pH6.8
Solution A – 28ml
Solution B – 72ml
Distilled water – 100ml
Adjust the pH

Final volume – 200ml

Toluidine blue 0.05% in Phosphate buffer 0.1M pH6.8
Toluidine blue – 0.05g
Phosphate buffer 0.1M pH6.8 – 100ml

Dissolve dye in buffer, stir for 30 min. Keep it at 4 degree Celsius.

Protocol for staining with Toluidine Blue

For fresh tissue sections.

1. Section plant and add sections to petri dish containing cold water
2. Immerse the slides in 5X diluted Toluidine blue: 12 seconds (10~30 seconds, dependent on plant, tissue, section thickness, and dye concentration.)
3. Wash slides in DI water for 1 min, repeat 3 times or until there is no more blue run off.
4. Wash dish with water and transfer sections to microscope slide.