Histochemistry analysis of tree stem section

Day 1 – Fix tissue

Make up the amount of 1 × phosphate buffered saline (PBS) you will need and adjust the pH to 11 with NaOH. Heat the solution to 60 to 70°C. Add 4% paraformaldehyde*. The paraformaldehyde should dissolve within a few minutes. Place solution on ice and when it has cooled to about 4°C, adjust the pH with H_2SO_4 to pH 7.5.

Dispense the paraformaldehyde solution into glass scintillation vials. Place vials on ice in a vacuum desiccator. Add freshly harvested tissue, such as stem section from 1 to 10th internode of young tree, to the vials. Close the desiccator and apply a vacuum to the samples until the paraformaldehyde solution starts to bubble. Hold the vacuum for 15 minutes and then release it slowly. Repeat this procedure until the tissue sinks. Replace the paraformaldehyde solution with fresh paraformaldehyde solution and shake gently overnight at 4°C.

*: Do this in a fume hood

Day 2 – Dehydrate tissue*

Solution	time	Number of changes
1 × PBS	30 minutes	two
30% ethanol	60 minutes	one
40% ethanol	60 minutes	one
50% ethanol	60 minutes	one
60% ethanol	60 minutes	one
**70% ethanol	60 minutes	one
85% ethanol	60 minutes	one
95% ethanol	overnight	one

*. all steps are done at 4°C with gentle shaking or rotating. A lab quake rotator works well

**. Tissue can be stored in 70% ethanol at 4°C for at least several months

Day 3 – Finish dehydration and begin embedding*

Solution	time	Number of changes
100% ethanol	30 minutes	two
100% ethanol	60 minutes	two
25% Histoclear, 75% ethanol	60 minutes	one
50% Histoclear, 50% ethanol	60 minutes	one
75% Histoclear, 25% ethanol	60 minutes	one
100% Histoclear	60 minutes	two
100% Histoclear + ¼ volume Paraplast Plus (paraffin) chips	Overnight (no shaking)	one

*. All steps are done at room temperature with gentle shaking or rotating

Day 4 – continue embedding

Place the vials at 42°C until the Paraplast chips melt completely.

Add another ¼ volume of chips and wait until completely melted.

Move vials to 60°C.

After several hours, replace the wax/Histoclear solution with freshly melted wax. Leave overnigh.

Day 5 – continue embedding

Replace old wax with fresh wax twice

Day 6 – continue embedding

Replace old wax with fresh wax twice

Day 7 – continue embedding

Replace old wax with fresh wax twice

Day 8 – place tissue in molds

Place tissue in molds. We use aluminum weigh boats (Fisher Scientific). Be careful to arrange tissue in the weigh boats so that it will be easy to cut the wax block into appropriate sized pieces and so that it will be easy to orient the tissue for sectioning.

Store paraffin blocks with embedded tissue at 4°C.

– Section

– Histochemistry for cellulose and lignin

Solution	time	Number of changes
100% Histoclear	10 minutes	two
100% ethanol	1-2 minutes	two
95% ethanol	1-2 minutes	one
90% ethanol	1-2 minutes	one
80% ethanol	1-2 minutes	one
70% ethanol	1-2 minutes	one
1% Safranin in 50% ethanol (Stain lignin to red color)	30 minutes	one
70% ethanol	dip	one
95% ethanol	Dips	four
0.25% fast green in 95% ethanol (Stain cellulose to green color)	5 seconds	one
100% ethanol	15 seconds or less	one
100% xylene	1 minute	one

Mounting

Section on slice can be mounted in mounting media, such as Damar-xylene solution, saturated solution of gum damar in xylene.