

**PREPARATION OF STAINED BOTANICAL SPECIMENS FROM SECTIONS  
IN WAX ON SLIDES.**

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This protocol uses Alcian Blue and Safranin stains. The Alcian Blue is a 1% solution in 2.5% acetic acid solution. The Safranin is 1% Safranin in 50% alcohol.

The sections must be dewaxed, hydrated, stained, dehydrated cleared and mounted.

Process and reagent	Time	Comments
<u>Dewax</u>		
Xylene I	> 10 mins	The period can be extended without problem to ensure removal of all wax.
Xylene II	> 10 mins	
<u>Hydration</u>		
Alcohol I	5 mins	This is to replace the xylene. The section becomes more visible.
Alcohol II	5 mins	
Alcohol 50%	5 mins	Starts hydrating the section.
Water	1 min	Move slide slowly up and down to bring the section into water.
<u>Staining I</u>		
Alcian Blue	20 - 30 mins.	
<u>Wash</u>		
Water Rinse 1	1 min	To remove any excess stain
Water Rinse II	Dip & reverse	
<u>Staining II</u>		
Safranin in 50% alcohol	5 - 10 mins	Can be left much longer if required e.g. overnight
<u>Wash</u>		
Water	1 min	To remove excess Safranin stain Agitate gently to assist wash.
Water Rinse	Dip & reverse	
<u>Differentiation</u>		
50% alcohol	As necessary	Agitate gently until the section starts to take on a clear blue in parts.
<u>Dehydration</u>		
Alcohol 1	Dip & reverse	The dehydration only takes seconds - too long could remove too much Safranin.
Alcohol 2	Dip & reverse	
<u>Clearing</u>		
Xylene 1		To remove any alcohol ready for mountant Slide and section should become clear and transparent. If milky return slide to the Alcohol 1 then 2 and then xylenes again.
Xylene 2		
<u>Mounting</u>		
		Mount in Numount, Canada Balsam etc.

It is important that Dewax xylenes and alcohols are kept solely for this purpose - the reagents used for dewaxing the slides must not be used for clearing since they will contain wax.