

Cresyl Violet Staining Protocol

*For unfixed tissue

Step	Time (min)
1. Load glass tray with slides	
2. Thaw frozen slides at room temperature	10 min.
3. Soak slides in 4% Formaldehyde solution (PH ~7.2)	15 min.
4. Soak in 100 % Ethanol (Etoh)	02 min.
5. Soak in 95% Ethanol	02 min.
6. Soak in 70% Ethanol	02 min.
7. Soak in 50 % Ethanol	02 min.
8. Dip in deionized H2O	quick
9. Soak in Cresyl Violet	10 min.?
10. Dip in deionized H2O	quick
11. Soak in 50% Ethanol	04 min.
12. Soak in 70% Ethanol	04 min.
13. Soak in 95% Ethanol	04 min.
14. Dip in 100% Ethanol	04 min.
15. Soak in Xylene	5 min.
a. Leave slides in xylene until ready To coverslip	

NOTES:

Move up and down a lot. **

Take it out of the differentiator when you feel it has gotten sufficiently lighter. ##

Make sure to take out twenty cover slips in the very beginning so you don't make a mess by grabbing them while your gloves are covered in paramount!

After 5 minutes is up take two to four slides out of the xylene at a time and let them sit under the hood for 30 secs. or so and wipe the backs of the slides on the diaper in the hood. After most of the cresyl blotches are off the slide you can start mounting your cover slips. (This step is only needed if the safeclear is dirty, if the safeclear is new don't worry about doing this.)

Put one larger line of paramount on the top and the bottom of the cover slip and a little line in the center (more paramount is better than not enough!). Gently put on the slide and tap air bubbles

away with the backside of your thumb. Putting slight pressure on the air bubbles should push them right out, there is no need to use a lot of force. Change gloves often to avoid getting the outside of the cover slips or the backside of the slides sticky with paramount.

Making Cresyl Violet and the Differentiator:

** **(Cresyl Violet)**-Add 1.25g (100%) cresyl violet acetate and 0.75 ml glacial acetic acid to 250 ml warm DH20, cool and filter.

(Differentiator)-2 ml glacial acetic acid in 200 ml 70% ethanol.