CRESYL VIOLET STAIN FOR NISSL BODIES

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Adapted from Powers and Clark, 1955*

This protocol has been optimised for use with frozen primate brain tissue (marmoset and macaque).

Tissue preparation steps

1. Transcardial perfusion with heparinised 0.1M phosphate buffered saline followed by 4% buffered paraformaldehyde.
2. Overnight post-fixation in 4% buffered paraformaldehyde.
3. Remove brain from skull and block for sectioning.
4. Cryoprotect block with 10%, 20%, 30% sucrose in buffered paraformaldehyde.
5. Tissue block frozen and sections cut at 40 or 50µm.
6. Tissue sections post-fixed in 4% buffered formalin for 1-2 weeks then mounted on gel-subbed slides out of warm buffered 0.5% gelatine solution. Dry down for 5-7 days before defatting as below.

Day 1 - defatting

Defat overnight in 50:50 chloroform and 100% ethanol (solution may be re-used until a heavy sediment develops)

Day 2 – defatting and staining

Rinse sections in 100% ethanol 5 min, then place into xylene for 3 hrs (xylene defatting solution may be re-used until a heavy sediment develops or it goes cloudy on contact with Ethanol)
(NB. In our experience, rat tissue doesn't need the defatting steps described above).
Take sections through

1. Absolute ethanol (5 min) (do not re-use for dehydration step 9 below)
2. Absolute ethanol (5 min) (do not re-use for dehydration step 9 below)
3. 95% ethanol (2 min) (do not re-use for dehydration step 9 below)

4. Continue to rehydrate sections by taking through a series of ethanol solutions (70%, 50%, 25%, 10%, distilled water x 2) 2 minutes each.
5. From distilled water, drain sections briefly on paper towel and place in filtered (use the toe of a stocking) cresyl violet @ 38-40^{o}C for 8-10 minutes (0.05% in distilled water, ph 3.76. Use glacial acetic acid or 0.2M sodium acetate to adjust pH) check after 5 minutes. Ideal staining is dark, but with cell layers visible through the high background.
6. Remove to running tap water until the water runs clear
7. Take the slides back through distilled water x 2, 10%, 25%, 50%, 70% ethanol for 1 minute each. Use the same set of dishes as for step 4 above.
8. Differentiate in acidified 70% alcohol (add 4-6 drops of glacial acetic acid to 200ml 70% ethanol). So that cell nuclei and cell layers are distinguishable and background is removed.

NB. If gentler differentiation is required, differentiate in 70% ethanol alone or add Milton** instead of glacial acetic acid.

9. Dehydrate in fresh ethanol (95%, then absolute x 2) 10 – 15 dips

    NB. For larger tissue sections (3" x 2" slides, 50µm), it may be necessary to add a 1:3 ETOH: XYLENE step before adding to pure xylene.

10. Place in Xylene x 2 (5 mins each) before attaching coverslips with DPX.

* Stain Technology 30(2): 83-88

** https://en.wikipedia.org/wiki/Milton_sterilizing_fluid