

Nissl Staining in Paraffin Sections

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Solutions

0.5% Cresyl Violet Solution:

Add 2.5 g cresyl violet acetate (Sigma C-5042) to 500 mL distilled water. Add 10 drops 10% acetic acid, and dissolve completely by mixing and filtering twice.

Diluted Acetic Acid:

Add 2-3 drops concentrated acetic acid to 100 mL dH₂O

Staining Protocol

-Deparaffinize sections by rinsing (3 min ea) in HistoClear (2x), 50% HistoClear/50% EtOH, 100% EtOH (2x), 95% EtOH, 70% EtOH, and 50% EtOH

-Rehydrate slides in PBS 5 min at RT

-Rinse slides in cresyl violet solution, 12 min at RT

-Rinse in dH₂O and immediately go to the next step

-Rinse (3 quick dips) in diluted acetic acid

-Rinse in dH₂O

-Rinse **quickly** in 50%, 70%, 95%, 100% EtOH series

-Rinse in HistoClear 2 min

-Rinse in HistoClear 5 min

-Add Permount (Fisher SP 15-100) at the edge of the slide and mount w/ coverslip. Remove bubbles by pressing the slide carefully. Allow Permount to set in a well-ventilated place for 12-24 hr at RT.

Notes:

Use cresyl violet that is as fresh as possible. Also, when making the solution, stir for at least 20-30 min under foil. Keep prepared solution under foil. When performing the staining, check slides after step 4. If your sections are too lightly stained, put them back into cresyl violet longer.