

Kluver-Barrera Method for Myelin and Nerve Cells

For Formalin Fixed Paraffin Sections

Solutions

0.1% Luxol Fast Blue

Luxol Fast Blue (Solvent Blue 38 Sigma).....0.1gm
Alcohol 95%.....100ml

Dissolve Dye in alcohol. Add 0.5ml of 10% Glacial Acetic Acid to each 100ml

0.1% Cresyl Echt Violet Solution

Cresyl Echt Violet.....0.1gm
Distilled Water.....100ml

Just before using, add 15 drops of 15% glacial acetic acid then Filter.

0.05% Lithium Carbonate Solution

Lithium Carbonate.....0.05gm
Distilled Water.....100ml

Staining Procedure

1. Deparaffinize and hydrate to 95% alcohol.
2. Luxol fast blue solution at 56^o-60^oC overnight.
3. Rinse in 95% alcohol to remove excess stain.
4. Rinse in distilled water.
5. Begin differentiation by quick immersion in lithium carbonate solution
6. Continue differentiation in 70% alcohol solution until gray and white matter can be distinguished.
7. Wash in distilled water.
8. Finish differentiation by rinsing briefly in lithium carbonate solution and then putting through several changes of 70% alcohol until the greenish blue of the white matter contrasts sharply with the colorless gray matter.
9. Rinse thoroughly in distilled water
10. Filter and preheat Cresyl echt violet to 57^oC just before use.
11. Cresyl echt violet solution for 6 minutes.
12. Differentiate in several changes of 95% alcohol (add a few drops of 5N HCL to speed up differentiation).
13. Dehydrate in absolute alcohol, and clear in xylene.

Results

Myelin—Blue Cell Products—Pink to Violet

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