

Pathology Core Protocol



Protocol Section: Staining Procedures-Manual
Protocol Subject: Luxol Fast Blue for Myelin

Policy No: P-CMHD2-LFB-01
Effective Date:
Date Reviewed: 6 January 2003
Date Revised: September 22, 2003

Fixation: 10% Formalin

Sections: Cut paraffin sections at 507 microns. Frozen or nitrocellulose sections may also be used.

Staining Solutions:

- a. 0.1% Solvent Blue in 95% alcohol – add 0.5 ml of 10% acetic acid per 100 ml of solution
- b. 0.5% lithium carbonate
- c. Mayer's haematoxylin – see P-CMHD2-HE
- d. Eosin – see P-CMHD2-HE

Staining Procedures:

- 1. Take section to 95% alcohol.
- 2. Stain in 0.1 LFB in 95% alcohol for 2 hours at 60°C.
- 3. Wash in water.
- 4. Differentiate with saturated lithium carbonate in water.
- 5. Wash in water.
- 6. Stain in Harris's haematoxylin for 5 minutes.
- 7. Wash in water, differentiate in acid alcohol 3 to 6 dips.
- 8. Wash well in water, blue in Scott's Tapwater substitute 1 minute.
- 9. Wash well in running water.
- 10. Rinse in 95% alcohol.
- 11. Stain 1 minute in eosin, making sure stain covers slides completely.
- 12. Wash well in running water.
- 13. Dehydrate in 95% alcohol and 3 changes of absolute alcohol, 10 dips in each.
- 14. Clear in 3 changes of xylene, 10 dips each, and mount.

Result:

Myelin sheaths – dark blue
Nuclei – blue-black
Background – pink

Reference: Ralis, Heather M. (1973) Techniques in Neurohistology. Butterworths.

Issued by Lab Manager: _____ **Date:** _____

Approved by Facility Management: _____ **Date:** _____