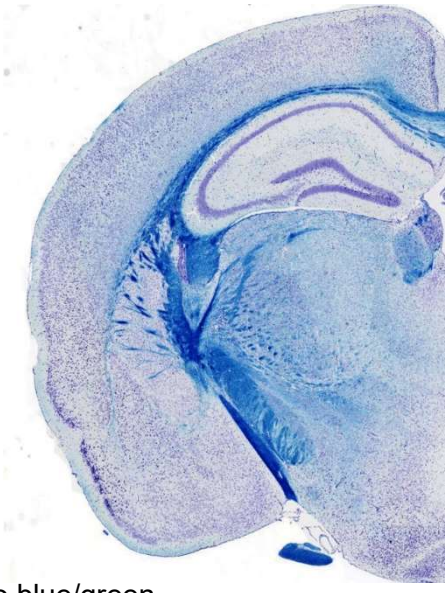
30 Minute Luxol Fast Blue Stain Kit

Description

The 30 Minute Luxol Fast Blue Stain Kit is designed for staining myelin/myelinated axons and Nissil substance on formalin fixed, paraffin-embedded tissue as well as frozen tissue. This product is used for identifying the basic neuronal structure in brain or spinal cord sections, and contains a Cresyl Etch Violet counterstain.



Staining without a microwave takes between 2-24 hours; we include a modified microwave procedure that substantially reduces staining time to 30 minutes.

Myelin (white matter)	Blue to blue/green
Gray matter and cytoplasm	Shades of pink to red
Nuclei	Dark blue

Kit Contents

Product	Volume	Storage
Cresyl Echt Violet Solution	125 ml	2-8°C
Luxol Fast Blue Solution	125 ml	18-25°C
Lithium Carbonate Solution (0.05%)	500 ml	18-25°C
Alcohol, Reagent (70%)	500 ml	18-25°C

Uses/Limitations

Not to be taken internally. This is for in-vitro diagnostic use only. Do not use if reagents become cloudy. Do not use past expiration date. Use caution when handling reagents.
Non-Sterile.

Control Tissue

This kit works best with formaldehyde-fixed paraffin embedded sections for staining myelinated issue such as the cerebral cortex and spinal cord.

Procedure

1. Deparaffinize sections thoroughly. We recommend three changes of xylene, 3 minutes each, but follow recommendations if using non-Xylene based deparaffinizer.
2. Hydrate tissue through two changes each of 100% and 95% ethyl alcohols, 10 dips each. **Critical Step: Do not rinse in distilled water after 95% ethanol.**
3. Drain rack to prevent solution carry over.
4. Incubate slides in Luxol Fast Blue Solution for 2 hours at 60°C or overnight at 37°C; seal lids tightly.

   If you wish to perform 30 minute staining, substitute this procedure:
   a. Place slides in a plastic Coplin jar containing Luxol Fast Blue Stain 0.1%,
   b. Using a laboratory grade microwave oven, microwave at 70°C for 10 minutes. (Although you can use a consumer microwave oven to achieve the same result, temperature control is difficult and can generate hot-spots and uneven staining.) S*taining conditions will vary based upon the oven used, so is a starting recommendation only. Observe proper precautions when using a microwave; operate in a fume hood to avoid exposure to fumes.*

5. Rinse slides quickly in 95% ethyl alcohol, 2-3 dips.
6. Rinse slides in distilled water.
7. Differentiate each slide individually (**critical step**). Immerse slide in Lithium Carbonate 0.05% for 20-30 seconds with agitation or until gray matter and demyelinated white matter are colorless and in high contrast with stained tissue. Save this solution for use in Step 10.
8. Continue differentiation in 70% ethyl alcohol, until gray and white matter can be distinguished. Observe the slide periodically; do not over differentiate.
9. Rinse slides in distilled water.

10. Complete differentiation; rinse slides briefly in Lithium Carbonate 0.05%, then rinse with two changes of 70% ethyl alcohol until the greenish/blue white matter sharply contrasts with the colorless gray matter.
11. Rinse thoroughly in distilled water.
12. Incubate slide in Cresyl Echt Violet Solution for 2-5 minutes.
13. Rinse quickly in 1 change of distilled water.
14. Dehydrate in two changes each of 95% and 100% ethyl alcohol. Clear in three changes of xylene, 10 dips each.
15. Coverslip with Xylene based compatible mounting medium.

References

1. Bancroft, John D., and Marilyn Gamble. Theory and Practice of Histological Techniques. 6th ed. Oxford: Churchill Livingstone Elsevier, 2008. 378.
2. Carson, Freida L., and Christa Hladik. Histotechnology: A Self-Instructional Text. 3rd ed. Chicago, Ill.: American Society of Clinical Pathologists, 2009. 212-219.
3. Klüver, Heinrich, and Elizabeth Barrera. "A Method for the Combined Staining of Cells and Fibers in the Nervous System." Journal of Neuropathology and Experimental Neurology 12.4 (1953): 400-403.
4. Luna, Lee G. Histopathologic Methods and Color Atlas of Special Stains and Tissue Artifacts. Gaitheresburg, MD: American Histolabs, 1992. 494-495.