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Part 12218
Revised March 2025

Luxol Fast Blue (LFB) Stain Set - Technical Memo

SET INCLUDES:	**Part 12218A**	**Part 12218B**
Solution A: Luxol Fast Blue Stain 0.1%, Alcoholic	500 ml	1000 ml
Solution B: Lithium Carbonate, Saturated Aqueous	500 ml	1000 ml

Additionally Needed For LFB/H&E Stain:	
Luxol Fast Blue (LFB) Control Slides	Part 4407
Hematoxylin Stain, Harris Modified	Part 1201
Acid Alcohol 1%	Part 10011
Eosin Y Working Solution	Part 1072
Xylene, ACS	Part 1445
Alcohol, Ethyl Denatured, 100%	Part 10841
Alcohol, Ethyl Denatured, 95%	Part 10842
Alcohol, Ethyl Denatured, 70%	Part 10844
Coplin Jar, Plastic	Part 5184 (for microwave modification)

For storage requirements and expiration date refer to individual product labels.

APPLICATION:

Newcomer Supply Luxol Fast Blue (LFB) Stain Set, with included microwave modification, is for the demonstration of myelin in central nervous system and peripheral nerve tissues.

The LFB Stain Set is flexible and can be used as a stand-alone without additional stain/counterstain or combined with options such as:

- LFB/Hematoxylin or LFB/Hematoxylin and Eosin (H&E)
- LFB/PAS or LFB/PAS/Hematoxylin
- LFB/Cresyl Violet
- LFB/Nuclear Fast Red
- LFB/Silver Nitrate

METHOD:

Fixation: Formalin 10%, Phosphate Buffered (Part 1090)
Technique: Paraffin sections cut at 8-10 microns on adhesive slides
Solutions: All solutions are manufactured by Newcomer Supply, Inc.

All Newcomer Supply Stain Sets are designed to be used with Coplin jars filled to 40 ml following the provided staining procedure. Some solutions in the set may contain extra volumes.

PRESTAINING PREPARATION:

1. If necessary, heat dry tissue sections/slides in oven.
2. Prepare Working Lithium Carbonate 0.05%; combine and mix well;
   a. *Solution B: Lithium Carbonate, Saturated Aqueous 5 ml*
   b. *Distilled Water 95 ml*

LFB/H&E STAINING PROCEDURE:

3. Deparaffinize sections thoroughly in three changes of xylene, 3 minutes each. Hydrate through two changes each of 100% and 95% ethyl alcohols, 10 dips each.
   a. *Stop at 95% ethyl alcohol; no distilled water rinse.*
   b. *See Procedure Notes #1 and #2.*
4. Incubate in Solution A: Luxol Fast Blue Stain 0.1%, Alcoholic for 2 hours at 60°C or overnight at 37°C; cover tightly.
   a. *To enhance stain, add 0.4 ml of Acetic Acid, Glacial, ACS (10010) to 40 ml of Solution A: Luxol Fast Blue Stain 0.1%, Alcoholic before use.*

   Microwave Modification: *See Procedure Note #3.*
   a. *Place slides in a plastic Coplin jar (5184) with Solution A: Luxol Fast Blue Stain 0.1%, Alcoholic. Microwave for 10 minutes at 70°C.*
5. Rinse slides quickly in 95% ethyl alcohol; 2-3 dips.
6. Rinse in distilled water.
7. Differentiate slides individually in Working Lithium Carbonate 0.05% (Step #2) for 10-15 seconds with agitation until gray matter and white matter are colorless and contrast with stained tissue.
8. Further differentiate in 70% ethyl alcohol, until gray and white matter can be distinguished. Do not over differentiate.
9. Rinse in distilled water.
10. Check slides microscopically. Continue if additional differentiation is needed. Otherwise proceed directly to Step #12.
    a. *One dip in Lithium Carbonate 0.05%, Aqueous (Step #2).*
    b. *Dip in two changes of 70% ethyl alcohol until green/blue white matter sharply contrasts with colorless gray matter.*
11. Rinse thoroughly in distilled water.
12. Stain with Hematoxylin Stain, Harris Modified (1201) for 1-5 minutes, depending on preference of intensity.
13. Wash in running tap water for 3 minutes.
14. Differentiate quickly in Acid Alcohol 1% (10011); 3 dips.
15. Wash well in running tap water.
16. Blue in Solution B: Lithium Carbonate, Saturated Aqueous.
17. Wash well in running tap water.
18. Counterstain in Eosin Y Working Solution (1072) for 30 seconds to 3 minutes, depending on preference of intensity.
19. Dehydrate in two changes of 95% for 1 minute each and two changes of 100% ethyl alcohol, 10 dips each. Clear in three changes of xylene, 10 dips each; coverslip with compatible mounting medium.

RESULTS:

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

PROCEDURE NOTES:

1. Drain slides after each step to prevent solution carry over.
2. Do not allow sections to dry out at any point during procedure.
3. The microwave procedure was tested using a laboratory-grade microwave oven. This procedure is a guideline and techniques should be developed for use in your laboratory.
4. If using a xylene substitute, follow manufacturer's recommendation for deparaffinization and clearing steps.

REFERENCES:

1. Carson, Freida L. and Christa Hladik Cappellano. *Histotechnology: A Self-instructional Text*. 4th ed. Chicago: ASCP Press, 2015. 206-211.
2. 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.
3. Luna, Lee G. *Histopathologic Methods and Color Atlas of Special Stains and Tissue Artifacts*. Gaitheresburg, MD: American Histolabs, 1992. 494-495.
4. Modifications developed by Newcomer Supply Laboratory.