

## Luxol Fast Blue (LFB) Stain Set - Technical Memo

### SET INCLUDES:

Solution A: Luxol Fast Blue Stain 0.1%, Alcoholic  
Solution B: Lithium Carbonate, Saturated Aqueous

Part 12218A	Part 12218B
500 ml	1000 ml
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 a commonly used procedure for the demonstration of myelin in central nervous system tissues and in peripheral nerve.

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

Staining results will vary according to option used.

### 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 staining procedure provided below. Some solutions in the set may contain extra volumes.

### LFB/H&E STAINING PROCEDURE:

1. 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.
  2. Incubate slides in Solution A: Luxol Fast Blue Stain 0.1%, Alcoholic for 2 hours at 60°C or overnight at 37°C; seal lids tightly.
    - a. To enhance staining and differentiation process for both regular and microwave modification, 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 containing Solution A: Luxol Fast Blue Stain 0.1%, Alcoholic and microwave at 70°C for 10 minutes.
3. Rinse slides quickly in 95% ethyl alcohol (10842); 2-3 dips.
  4. Rinse in distilled water.
  5. Prepare Working Lithium Carbonate 0.05%; combine and mix well.
    - a. Solution B: Lithium Carbonate, Saturated Aqueous 2 ml
    - b. Distilled Water 38 ml
  6. Differentiate each slide individually in Working Lithium Carbonate 0.05% for 10-20 seconds with agitation until gray matter and white matter are colorless and in high contrast with stained tissue.
    - a. Save solution for reuse in Step #9a.

7. Further differentiate in 70% ethyl alcohol (10844), until gray and white matter can be distinguished. Do not over differentiate.
8. Rinse distilled water.
9. Complete differentiation:
  - a. Rinse slides briefly in Working Lithium Carbonate 0.05%.
  - b. Rinse in two changes of 70% ethyl alcohol until green/blue white matter contrasts with colorless gray matter.
10. Rinse thoroughly in distilled water.
11. Stain with Hematoxylin Stain, Harris Modified (1201) for 1-5 minutes, depending on preference of stain intensity.
12. Wash in running tap water for 3 minutes.
13. Differentiate quickly in Acid Alcohol 1% (10011); 3 dips.
14. Wash well in running tap water.
15. Blue slides in Solution B: Lithium Carbonate, Saturated Aqueous.
16. Wash well in running tap water.
17. Counterstain in Eosin Y Working Solution (1072) for 30 seconds to 3 minutes, depending on preference of stain intensity.
18. 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 suggested microwave procedure has been tested at Newcomer Supply. This procedure is a guideline and techniques should be developed for use in your laboratory.
4. If using a xylene substitute, closely follow the manufacturer's recommendations 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*. Gaithersburg, MD: American Histolabs, 1992. 494-495.
4. Modifications developed by Newcomer Supply Laboratory.