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Part 12218
Revised April 2017

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 a commonly used procedure for the demonstration of myelin in central nervous system tissues and in peripheral nerve.

The Luxol Fast Blue (LFB) Stain Set has flexible uses and can be used as a stand-alone set without the inclusion of any other stain/counterstain or can be combined with staining options such as:

- LFB/Hematoxylin and Eosin (H&E)
- LFB/Hematoxylin
- 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
- Air-dry for a minimum of 30 minutes

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.
   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 slides in distilled water.
5. Prepare Working Lithium Carbonate 0.5%; combine and mix well.
   a. *Solution B: Lithium Carbonate, Saturated Aqueous 20 ml*
   b. *Distilled Water 20 ml*
6. Differentiate each slide individually; immerse in Working Lithium Carbonate 0.5% for 20-30 seconds with agitation or until gray matter and demyelinated white matter are colorless and in high contrast with stained tissue.
   a. *Save solution for reuse in Step #9a.*
7. Continue differentiation in 70% ethyl alcohol (10844), until gray and white matter can be distinguished. Do not over differentiate.
8. Rinse slides in distilled water.
9. Complete differentiation:
   a. *Rinse slides briefly in Working Lithium Carbonate 0.5%.*
   b. *Rinse in two changes of 70% ethyl alcohol until greenish/blue white matter sharply 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 staining rack/slides after each step to prevent solution carry over.
2. Do not allow sections to dry out at any point during staining procedure.
3. The suggested microwave procedure has been tested at Newcomer Supply using an “EB Sciences”, 850 watt microwave oven with temperature probe and agitation tubes. This procedure is reproducible in our laboratory. It is nonetheless a guideline and techniques should be developed for your laboratory which meet the requirements of your situation. Microwave devices should be placed in a fume hood or vented into a fume hood, according to manufacturer’s instructions, to prevent exposure to chemical vapors.
4. If using a xylene substitute, closely follow the manufacturer’s recommendations for deparaffinization and clearing steps.

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 Cappellano. *Histotechnology: A Self-instructional Text*. 4th ed. Chicago: ASCP Press, 2015. 206-211.
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.
5. Modifications developed by Newcomer Supply Laboratory.