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Part 4407
Revised January 2020

Luxol Fast Blue (LFB) Control Slides – Technical Memo

CONTROL SLIDES:

	Part 4407A	**Part 4407B**
	10 Slide/Set	98 Slide/Set

PRODUCT SPECIFICATIONS:
Tissue: Positive staining spinal cord.
Fixation: Formalin 10%, Phosphate Buffered (Part 1090).
Section/Glass: Paraffin sections cut at 8 microns on Superfrost™ Plus slides.
Quality Control Stain: Luxol Fast Blue quality control stained slide(s) included.
Reactivity: Guaranteed product specific reactivity for one year from date of receipt. Revalidate after one year to verify continued reactivity.
Storage: 15-30°C in a light deprived and humidity controlled environment.
Intended Use: To verify histological techniques and reagent reactivity.

Before using unstained control slides, review the enclosed stained slide(s) to ensure that this tissue source is acceptable for testing needs.

CONTROL SLIDE VALIDATION:

With Luxol Fast Blue (LFB) Stain Set:	**Part 12218A/B**	**Individual Stain Solution**
Solution A: Luxol Fast Blue Stain 0.1%, Alcoholic	500/1000 ml	
Solution B: Lithium Carbonate, Saturated Aqueous	500/1000 ml	Part 12215
Hematoxylin Stain, Harris Modified		Part 1201
Acid Alcohol 1%		Part 10011

APPLICATION:

Newcomer Supply Luxol Fast Blue (LFB) Control Slides are for the positive histochemical staining of myelin sheath in central nervous system tissue and in peripheral nerve.

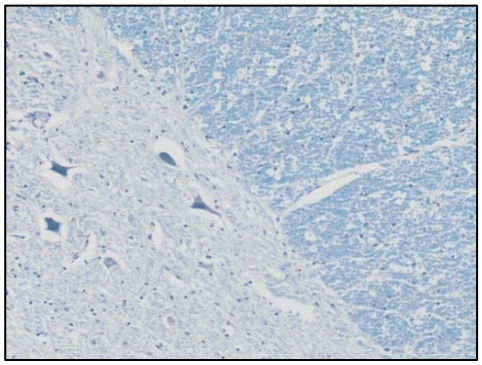
Periodic Acid Schiff (PAS), cresyl violet, hematoxylin or eosin stains/counterstains can be added to the LFB procedure for enhanced staining.

NEWCOMER SUPPLY VALIDATION PROCEDURE:

1. Heat dry sections in oven according to your laboratory protocol.
2. 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.*
3. Incubate 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 (5184) containing Solution A: Luxol Fast Blue Stain 0.1%, Alcoholic and microwave at 70°C for 10 minutes.*
4. Rinse slides quickly in 95% ethyl alcohol; 2-3 dips.
5. Rinse slides in distilled water.
6. Prepare Working Lithium Carbonate 0.5%; combine and mix well.
   a. *Solution B: Lithium Carbonate, Saturated Aqueous 20 ml*
   b. *Distilled Water 20 ml*
7. Differentiate each slide individually; immerse slide in Working Lithium Carbonate 0.5%, Aqueous for 20-30 seconds.
   a. *Save solution and reuse in Step #10a.*
8. Continue differentiation in 70% ethyl alcohol (10844), until gray and white matter can be distinguished. Do not over differentiate.
9. Rinse slides in distilled water.
10. Complete differentiation:
    a. *Rinse briefly in Working Lithium Carbonate 0.5%, Aqueous.*
    b. *Rinse in two changes of 70% ethyl alcohol until greenish/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 stain 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. Dehydrate in two changes each of 95% and 100% ethyl alcohol. Clear in three changes of xylene, 10 dips each; coverslip with compatible mounting medium.

RESULTS:

Myelin	Blue
Nissl substance and nuclei	Blue
Other tissue components	Dependent on other stains used



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. 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. Modifications developed by Newcomer Supply Laboratory.

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