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Part 4407
Revised May 2017

Luxol Fast Blue (LFB) Control Slides – Technical Memo

CONTROL SLIDES: **Part 4407A** 10 Slide/Set **Part 4407B** 98 Slide/Set

Luxol Fast Blue (LFB) Control Slides contain a section of positive staining spinal cord.

PRODUCT DESCRIPTION:
The enclosed positive control slides are intended to be used to verify histological techniques and reagent reactivity. These slides are to be used for the qualitative purpose of determining positive or negative results, and are not intended to be used for any quantitative purpose. The first serial section within the control box is stained and provided for your reference. ***Before using the unstained slides, review the enclosed stained slide with your pathologist to ensure that this tissue source is acceptable. Newcomer Supply will not accept a return with missing slides in the series. Newcomer Supply guarantees reactivity of these control slides for one year from the date of receipt. Revalidate after one year to verify continued reactivity. Store at 15-30°C in a light deprived and humidity controlled environment.***

These positive control slides were produced from human surgical or autopsy tissues under carefully controlled conditions. Reasonable measures are used to deliver quality control slides that are as consistent as possible. However, characteristics of quality control slides may be dissimilar due to variations in the reagents, stains, techniques, laboratory conditions, and tissue sources used. Newcomer Supply Laboratory uses a manual method of performing quality control procedures, specifically avoiding automation, in order to provide reactive control slides for even less aggressive methods of staining that our customers may be using.

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

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

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 additionally enhanced staining.

METHOD:

Fixation: Formalin 10%, Phosphate Buffered (Part 1090)
Technique: Paraffin sections cut at 8 microns on Superfrost® Plus
- Air-dry for a minimum of 30 minutes

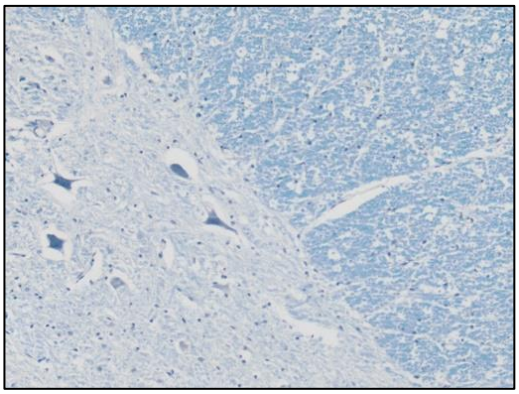
Solutions: All solutions are manufactured by Newcomer Supply, Inc.

NEWCOMER SUPPLY VALIDATION 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 (page 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 (page 2).*
   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.*
3. Rinse slides quickly in 95% ethyl alcohol; 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 slide in Working Lithium Carbonate 0.5%, Aqueous for 20-30 seconds.
   a. *Save solution and 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 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.*
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. 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 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. Modifications developed by Newcomer Supply Laboratory.