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Part 9155
Revised December 2015

Luxol Fast Blue (LFB) - Cresyl Violet Stain Kit - Technical Memo

KIT INCLUDES:	**Part 9155A**
Solution A: Luxol Fast Blue Stain 0.1%, Alcoholic	250 ml
Solution B: Lithium Carbonate 0.5%, Aqueous	250 ml
Solution C: Cresyl Violet Stain, Aqueous	250 ml
Solution D: Acetic Acid 10%, Aqueous	50 ml

COMPLIMENTARY POSITIVE CONTROL SLIDES: Enclosed with this kit are two complimentary unstained positive control slides to be used for the initial verification of staining techniques and reagents. Verification must be documented by running one Newcomer Supply complimentary positive control slide along with your current positive control slide for the first run. Retain the second complimentary control slide for further troubleshooting, if needed.

Individual stain solutions and additional control slides may be available for purchase under separate part numbers at www.newcomersupply.com.

Additionally Needed:

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 bottle labels.

APPLICATION:

Newcomer Supply Luxol Fast Blue (LFB) - Cresyl Violet Stain Kit, with included microwave modification, is a commonly used procedure for the demonstration of myelin and Nissl substance in central nervous system tissues and in peripheral nerve.

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 Kits are designed to be used with Coplin jars filled to 40 ml following the staining procedure provided below. Some solutions in the kit may contain extra volumes.

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, 2-3 dips.
4. Rinse slides in distilled water.
5. Differentiate each slide individually; immerse slide in Solution B: Lithium Carbonate 0.5%, Aqueous for 20-30 seconds with agitation.
    a. *Save solution and reuse in Step #8.*
6. Continue differentiation in 70% ethyl alcohol, until gray and white matter can be distinguished. Do not over differentiate.
7. Rinse slides in distilled water.
8. Complete differentiation; rinse slides briefly in Solution B: Lithium Carbonate 0.5%, Aqueous, then rinse in two changes of 70% ethyl alcohol until greenish/blue white matter sharply contrasts with colorless gray matter.
9. Rinse thoroughly in distilled water.
10. Prepare Cresyl Violet Stain Working Solution:
    a. Solution C: Cresyl Violet Stain, Aqueous 40 ml
    b. Solution D: Acetic Acid 10%, Aqueous 7 drops
    c. Combine, mix well and filter.
    d. Directly before use, preheat filtered solution to 57°C in microwave; hold in oven.
11. Stain in preheated Cresyl Violet Stain Working Solution for 6 minutes, keeping solution warm in oven during staining.
12. Rinse in distilled water.
13. Dehydrate quickly in two changes each of 95% and 100% ethyl alcohol. Clear in three changes of xylene, 10 dips each; coverslip with compatible mounting medium.
    a. See Procedure Note #4.

RESULTS:

Myelin	Blue
Nissl substance and nuclei	Violet
Neurons	Pink to violet

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. Dehydrate quickly to maintain Cresyl Violet staining.
5. 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. *Histotechnology: A Self-Instructional Text*. 3rd ed. Chicago, Ill.: American Society of Clinical Pathologists, 2009. 214-215.
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.