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Part 1039
Revised November 2019

Cresyl Violet Acetate Stain for Nerve Tissue - Technical Memo

SOLUTION:	**100 ml**
Cresyl Violet Acetate 2.5%, Aqueous	Part 1039A

Additionally Needed:

Luxol Fast Blue (LFB) Control Slides	Part 4407
Luxol Fast Blue (LFB) Stain Set	Part 12218 (for LFB/Cresyl Violet Acetate Stain)
Acetic Acid, Glacial, ACS	Part 10010
Xylene, ACS	Part 1445
Alcohol, Ethyl Denatured, 100%	Part 10841
Alcohol, Ethyl Denatured, 95%	Part 10842

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

APPLICATION:

Newcomer Supply Cresyl Violet Acetate 2.5%, Aqueous is a metachromatic basic dye for the demonstration of Nissl substance and nuclei in nerve tissue. As a working solution, Cresyl Violet Acetate can be utilized in the Luxol Fast Blue (LFB) procedure or used solely as a stand-alone nerve tissue stain.

Cresyl Violet Acetate is also known as Cresyl Violet, Cresyl Fast Violet Acetate and Cresyl Echt Violet Acetate. Due to characteristics of the dye, the actual percentage of dye concentration in Cresyl Violet powder may vary between lots. The minimum acceptable dye content for Cresyl Violet certification is noted as 65%.

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.

STAINING PROCEDURES:

1. If necessary, heat dry tissue sections/slides in oven.
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. *LFB procedure: stop at 95% ethyl alcohol, no distilled water rinse.*
    b. *Cresyl Violet Acetate stand-alone staining: continue with distilled water rinse. Proceed to Step #6.*
    c. *See Procedure Notes #1 and #2.*
3. LFB Stain: proceed with LFB Stain Set (12218) protocol through final differentiation step and rinse thoroughly in distilled water.
4. Continue with LFB counterstain or Cresyl Violet Acetate stand-alone staining.
5. Prepare Acetic Acid 10%, Aqueous; combine and mix well.
    a. *Acetic Acid, Glacial, ACS (10010) 10 ml*
    b. *Distilled Water 90 ml*
    c. *Store at room temperature for up to 1 year.*
6. Prepare Working Cresyl Violet Acetate 0.25%; mix well and filter.
    a. *Cresyl Violet Acetate 2.5%, Aqueous 5 ml*
    b. *Distilled Water 45 ml*
    c. *Acetic Acid 10%, Aqueous 7 drops*
    d. *Directly before use, preheat filtered solution to 57°C in microwave; hold in oven.*
    e. *See Procedure Notes #3 and #4.*
7. Stain in filtered, preheated Working Cresyl Violet Acetate 0.25% for 6 minutes.
    a. *Keep solution warm in oven during staining.*
8. Rinse in distilled water.
9. 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 #5.*

RESULTS:

LFB with Cresyl Violet:	Myelin	Blue
	Nissl substance and nuclei	Violet
	Neurons	Pink to violet
Cresyl Violet alone:	Nissl substance	Purple/dark blue
	Nuclei	Purple blue
	Neurons	Pale purple-blue
	Background	Colorless

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. Due to possible variance of dye percentage between lots of Cresyl Violet powder, a stronger solution of Working Cresyl Violet Acetate may be required for optimal staining results. Test each new lot of Cresyl Violet Acetate 2.5%, Aqueous to determine best working stain concentration.
4. For improved staining of Working Cresyl Violet Acetate, adjust pH to 4.0 with Acetic Acid 10%, Aqueous.
5. Dehydrate quickly to maintain Cresyl Violet Acetate staining.
6. 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. 366-367.
2. Carson, Freida L., and Christa Hladik Cappellano. *Histotechnology: A Self-instructional Text*. 4th ed. Chicago: ASCP Press, 2015. 191-193, 207-208.
3. Horobin, Richard and John Kiernan. *Conn's Biological Stains: A Handbook of Dyes, Stains and Fluorochromes for Use in Biology and Medicine*. 10th ed. Oxford: BIOS, 2002. 281-282.
4. Modifications developed by Newcomer Supply Laboratory.