PRINCIPLE:  This stain demonstrates myelin sheath and Nissl substance.

SPECIMEN:  Any well fixed paraffin embedded tissue cut at 10 microns.

QUALITY CONTROL:  American MasterTech Recommended Control Slide:
Cerebellum, CSC0825P

PROCEDURE:
1. Deparaffinize slide with Xylene or a Xylene Substitute and hydrate through alcohols.
2. Rinse slide in Distilled water.
3. Preheat Luxol Fast Blue Stain to 60°C in a waterbath, then place slide into stain and incubate for 1 hour.
4. Rinse slide in Distilled water.
5. Begin differentiating gray and white matter by quickly dipping slide several times in 0.05% Lithium Carbonate.
6. Continue differentiation by immersing slide through 2 changes of 70% Reagent Alcohol until the gray and white matter is clearly distinguished; nuclei should be colorless and myelin should be turquoise against a pale blue-gray background.
7. Rinse slide in Distilled water.
8. Place slide in Cresyl Echt Violet Stain for 10 minutes.
9. Rinse slide in Distilled water for 5 to 10 seconds.
10. Differentiate section by dipping slide 5 to 10 times in 70% REAGENT ALCOHOL.
11. Dehydrate slide quickly through 3 changes of fresh Absolute Alcohol; excess time will decolorize C.E.V. Stain.
12. Clear slide through 3 changes of fresh Xylene or a Xylene Substitute.
13. Coverslip using a permanent mounting media.

RESULTS:
- Myelin fibers: **BLUE to TURQUOISE**
- Nissl Substance: **DARK BLUE**
- Nuclei: **BLUE**


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