**Classic Luxol Fast Blue Staining procedure**

**Description:** Stains myelin/myelinated axons on formalin-fixed, paraffin-embedded brain and spinal cord tissue sections, and cryosections. Commonly used to identify basic neuronal structure in brain or spinal cord tissue.

**Fixation:** 10% formalin

**Sectioning:** Paraffin sections at 5-10 um. Frozen sections at 20-30um

**Solutions and Reagents:**

0.1% Luxol fast blue solution:

Luxol fast blue, MBS -------------------------- 0.1 g

Ethyl Alcohol, 95% ---------------------------- 100 ml

Glacial acetic acid ---------------------------- 0.5 ml

0.1% Cresyl echt violet solution:

Cresyl echt violet (cresyl fast violet) ------- 0.1 g

Distilled water -------------------------------- 100 ml

Add 10 drops of glacial acetic acid just before use and filter.

0.05% Lithium carbonate solution:

Lithium carbonate ------------------------------- 0.05 g

Distilled water ----------------------------------- 100 ml

**Protocol**

1. Dewax in Xylene – 2 mins
2. Dewax in Xylene – 2 mins
3. Dewax in Xylene – 2 mins
4. Wash in Absolute Alcohol – 2 mins
5. Wash in Absolute Alcohol – 2 mins
6. Wash in 95% Alcohol – 2 mins
7. Stain in Luxol Fast Blue -- 60 mins or until desired staining (no longer than 16 hours)
8. Rinse in 95% Alcohol – 1 min
9. Wash in 95% Alcohol – 2 mins
10. Differentiate in 0.05% Lithium Carbonate in distilled water – 30 secs
11. Rinse in Distilled Water- 1 min
12. Wash in 70% Alcohol – 30 secs
13. Wash in 70% Alcohol – 30 secs
14. Rinse in Distilled Water – 2 mins view under microscope
15. Wash in 90% Alcohol – 2 mins
16. Wash in Absolute Alcohol – 2 mins
17. Wash in Absolute Alcohol – 2 mins
18. Wash in Absolute Alcohol – 2 mins
19. Fix in Xylene – 2 mins
20. Fix in Xylene – 2 mins
21. Fix in Xylene – 2 mins
22. Mount slides with coverslips using DePeX

**Results**

Myelin (including phospholipids) -------- blue to green