



## VitroView™ Luxol Fast Blue Kit

Cat. No. VB-3006

### Introduction

This kit is used for staining of myelin/myelinated axons on formalin-fixed, paraffin-embedded brain and spinal cord tissue sections, as well as frozen sections. Myelin, including phospholipids, will stain blue to green, and neurons will stain violet. This stain is commonly used for identifying the basic neuronal structure in brain or spinal cord tissue.

### Kit Components

1. Luxol Fast Blue Solution - 250 ml
2. Cresyl Violet Solution - 250 ml
3. Lithium Carbonate Solution - 250 ml

### Storage

Room temperature.

### Protocol

1. Deparaffinize in Xylene I for 6 minutes and II for 6 minutes.
2. Rehydrate
  - a. Ethanol 100% (2 minutes)
  - b. Ethanol 100% (2 minutes)
  - c. Ethanol 95% (2 minutes)
  - d. Ethanol 95% (2 minutes)
3. Deparaffinize and hydrate to 95% ethyl alcohol (Frozen/vibratome sections may need to undergo a de-fat step: Place sections directly into 1:1 alcohol/chloroform for a few hours/overnight, then re-hydrate with 95% ethyl alcohol).
4. Leave in luxol fast blue solution in a 56° C oven overnight (for frozen sections, not longer than 16 hours).
5. Rinse off excess stain with 95% ethyl alcohol.
6. Rinse in distilled water.
7. Differentiate the slides in the lithium carbonate solution for 30 seconds.
8. Continue differentiation in 70% ethyl alcohol for 30 seconds.
9. Rinse in distilled water.
10. Check microscopically to see if gray matter is clear, and that white matter is sharply defined.
11. Repeat the differentiation steps (step 7-9) if necessary.
12. When differentiation is complete, place in distilled water.
13. Counterstain in the Cresyl Violet Solution for 30-40 seconds.
14. Rinse in distilled water. DO NOT rinse in 70% alcohol, which will clear the fast blue staining).
15. Differentiate the slides in 95% ethyl alcohol for 5 minutes (check microscopically).
16. Wash in 100% alcohol 2x5 min. Clear with 3 changes of xylene (5 minute per change)
17. Mount coverslip onto glass slide with Permount or some other suitable organic mounting medium.

### Results

- Myelin, including phospholipids ----- blue to green
- Neurons ----- pink to violet

**Positive Controls**

Brain, spinal cord.

**Precautions**

Handle with care. Avoid contact with eyes, skin and clothing. Do not ingest. Wear gloves.