

# Beaumont

Origination 11/3/2022  
Last 11/3/2022  
Approved  
Effective 11/3/2022  
Last Revised 11/3/2022  
Next Review 11/2/2024

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Histology  
Applicability Royal Oak

## Histology Special Stain - Luxol Fast Blue - Royal Oak

Document Type: Procedure

### I. PURPOSE AND OBJECTIVE:

The purpose of this document is to provide a procedure for the demonstration of myelin that insulates and protects nerve fibers. When an axon degenerates, the myelin sheath breaks down into simpler lipids, which is then removed.

### II. PRINCIPLE:

The reaction over-stains the tissue, and then differentiates the stain out of the tissue. It uses a dye, Luxol fast blue, which contains copper, making it blue. Luxol fast blue will stain the lipoproteins in the myelin sheath by an acid-base reaction with salt formation. The base of the lipoprotein replaces the base of the dye.

### III. SPECIMEN COLLECTION AND HANDLING:

- A. Fixation
  - 1. Any well-fixed tissue.
  - 2. 10% neutral buffered formalin preferred.
- B. Processing
  - 1. Standard processing.
- C. Section Thickness
  - 1. Routine specimens-10µm.
- D. Slide Drying

1. 60 minutes at 60°C.

E. Type of slide

1. Plain slides

## IV. REAGENTS:

A. 0.1% Luxol Fast Blue

|                                  |                 |
|----------------------------------|-----------------|
| <b>Luxol fast blue</b>           | <b>0.1 gm</b>   |
| <b>95% ethanol</b>               | <b>100.0 mL</b> |
| <b>Acetic acid, concentrated</b> | <b>0.5 mL</b>   |

1. Dissolve together Luxol fast blue and 95% ethanol.
2. Add acetic acid slowly, drop by drop.
3. Store at room temperature.
4. Stable for months.
5. May be re-used.

B. 0.05% Lithium Carbonate

|                          |                  |
|--------------------------|------------------|
| <b>Lithium carbonate</b> | <b>0.25 g</b>    |
| <b>Distilled water</b>   | <b>500.00 mL</b> |

Dissolve together. Store at room temperature. Stable for months.

C. 5% Aluminum Sulfate

|                         |                 |
|-------------------------|-----------------|
| <b>Aluminum sulfate</b> | <b>5.0 gm</b>   |
| <b>Distilled water</b>  | <b>100.0 mL</b> |

Dissolve together. Store at room temperature. Stable for months.

D. Nuclear Fast Red (may use vendor pre-made solution)

|                            |                 |
|----------------------------|-----------------|
| <b>Nuclear fast red</b>    | <b>0.1 gm</b>   |
| <b>5% aluminum sulfate</b> | <b>100.0 mL</b> |

Dissolve together with the aid of gentle heat. Cool. Filter. Add a few crystals of thymol. Store at room temperature or in refrigerator. Stable for months.

## V. EQUIPMENT:

- A. Balance
- B. 60°C oven

## VI. SUPPLIES:

- A. Balance
- B. Erlenmeyer flasks
- C. Graduated cylinders
- D. Acid clean coplin jars
- E. Forceps

## VII. QUALITY CONTROL:

Use a section of brain medulla or peripheral nerve as a positive control.

## VIII. SPECIAL SAFETY PRECAUTIONS:

- A. Luxol Fast Blue
  - 1. No hazards listed.
- B. Acetic Acid
  - 1. Add drop by drop to solution.
  - 2. May cause skin and eye burns.
- C. Lithium Carbonate
  - 1. Is toxic and an irritant.
  - 2. Harmful if swallowed.

## IX. PROCEDURE:

| Step | Action                                                                                | Time              | Notes                    |
|------|---------------------------------------------------------------------------------------|-------------------|--------------------------|
| 1    | Deparaffinize and hydrate slides through graded alcohol to distilled water.           |                   |                          |
| 2    | Place Luxol fast blue in 60°C oven to pre-warm.                                       | 1 hour            |                          |
| 3    | Remove stain from oven.                                                               |                   |                          |
| 4    | Place sections in 0.1% Luxol fast blue.                                               | 1-2 hours         |                          |
| 5    | Rinse in 95% ethanol to remove excess stain, 2 changes.                               | 5-10 seconds each |                          |
| 6    | Rinse in distilled water, 2 changes.                                                  | 5-10 seconds each |                          |
| 7    | Differentiate slides in 0.05% lithium carbonate.                                      | 10-20 seconds     |                          |
| 8    | Rinse in distilled water, 2 changes.                                                  | 5-10 seconds each |                          |
| 9    | Differentiate slides in 70% ethanol until gray and white matter can be distinguished. |                   | Use microscope to check. |
| 10   | Rinse in distilled water, 2 changes.                                                  | 5-10 seconds each |                          |

|    |                                                                                                                         |             |                          |
|----|-------------------------------------------------------------------------------------------------------------------------|-------------|--------------------------|
| 11 | Repeat steps E, F, G and H until blue-stained myelinated white matter contrasts sharply with the colorless gray matter. |             | Use microscope to check. |
| 12 | Wash in running tap water.                                                                                              | 1 minute    |                          |
| 13 | Counterstain in Nuclear Fast Red.                                                                                       | 1-5 minutes |                          |
| 14 | Dehydrate through graded alcohols.                                                                                      |             |                          |
| 15 | Clear in two changes of xylene.                                                                                         |             |                          |
| 16 | Coverslip.                                                                                                              |             |                          |

## X. LIMITATIONS:

- A. Use the microscope to differentiate.
- B. As the stain will be differentiated, the slides can remain in the solution longer than stated.
- C. Slides may be left in a 37°C oven overnight in the Luxol fast blue.
- D. If over-decolorized, place slides back in Luxol fast blue, then back into the 60°C oven for 1 hour,
- E. Repeat the differentiation step.
- F. A counterstain may be used such as PAS, cresyl echt violet, or H&E.

## XI. RESULTS:

- A. Myelin sheath - **blue-green to blue**
- B. Background - **pale pink**

## XII. REFERENCES:

- A. Bancroft JD and Stevens A: Theory and Practice of Histological Techniques, New York, New York, Churchill Livingstone, 1990
- B. Carson FL: Histotechnology: A Self-Instructional Text, Chicago, IL, ASCP Press, 1990.
- C. Sheehan DC, Hrapchak BB: Theory and Practice of Histotechnology, 2nd edition. Columbus, Ohio, Battelle Press, 1980.

## Approval Signatures

| Step Description | Approver                                        | Date      |
|------------------|-------------------------------------------------|-----------|
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## Applicability

Royal Oak

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