
LUXOL FAST BLUE - MODIFIED KLUVER'S - MYELIN SHEATH

PURPOSE: Myelin sheath is the white fatty non living material forming an insulating and protective sheath around nerve fibers.

PRINCIPLE: Luxol Fast Blue is the alcohol soluble counterpart of the water soluble Alcian Blue. Staining is due to lipoproteins, and the mechanism is one of an acid-base reaction with salt formation; the base of the lipoprotein replaces the base of the dye.

CONTROL: Normal cerebral cortex

FIXATION: 10% formalin

TECHNIQUE: Cut paraffin sections 10 μ . Allow to dry in a 37°C oven overnight.

EQUIPMENT: Rinse all glassware in DI water, coplin jars, 60°C oven.

REAGENTS:

Luxol Fast Blue Solution:

Luxol fast blue	1.0 gm
95% alcohol	100.0 ml
10% acetic acid	5.0 ml

Mix, filter. Solution is stable for 1 year.

CAUTION: Flammable liquid, avoid contact and inhalation.

0.05% Lithium Carbonate:

Lithium carbonate	0.5 gm
Distilled water	1000.0 ml

CAUTION: Avoid contact and inhalation.

Eosin Y Solution

0.25% Cresyl Echt Violet:

Cresyl Echt violet	0.25 gm
Distilled water	100.0 ml
10% Acetic acid	1.0 ml

Mix, filter. Stable for 2 years

CAUTION: Avoid contact and inhalation.

SAFETY: Wear gloves, goggles and lab coat. Work in a well ventilated area, avoid contact and inhalation.

Acetic acid; moderately toxic on ingestion. Target organ effects on respiratory system on inhalation. Irritant to skin, eyes and respiratory system.

Lithium carbonate; irritant to eyes and skin.

Alcohol; skin and eye irritant. Ingestion can cause gastrointestinal irritation, nausea, vomiting diarrhea, blindness, death. Excessive inhalation can cause nasal and respiratory irritation.

PROCEDURE:

1. Deparaffinize and hydrate to 95% alcohol.
2. Luxol fast blue solution, overnight, 60°C .
3. Rinse in 95% alcohol.
4. Rinse in distilled water.
5. Lithium carbonate solution, 5 seconds.
6. 70% alcohol, two changes, 10 seconds each.
7. Wash in distilled water.
8. Repeat steps 5 - 7 until there is a sharp contrast between the blue of the white-matter and the colorless gray-matter.
9. Rinse slides in 70% alcohol.
10. Eosin, 1 minute.
11. Rinse in distilled water.
12. Cresyl violet, 1 minute.
13. Rinse in distilled water.
14. Dehydrate through 95% and 100% alcohol, clear in xylene, and coverslip.

RESULTS:

Myelinated fibers	blue
Neurophils	pink
Nerve cells	purple

REFERENCES:

Sheehan D, Hrapchak B, Theory and practice of Histotechnology, 2nd ed,1980, pp262-264, Battelle Press, Ohio
Crookham,J, Dapson,R, Hazardous Chemicals in the Histopathology Laboratory, 2nd ED, 1991, Anatech

Prepared: _____ By: _____

Approved: _____ By: _____

PROCEDURE CARD

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Distilled water 1000.0 ml

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DATE: _____

TECH: _____

EXPIRATION: _____

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LUXOL FAST BLUE

DATE: _____

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EOSIN

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0.25% CRESYL VIOLET

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