

Luxol Fast Blue Stain Kit

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Intended Use

For In Vitro Diagnostic Use

Summary and Explanation

The Luxol Fast Blue Stain Kit is designed for staining myelin/myelinated axons and Nissl substance on formalin fixed, paraffin-embedded tissue as well as frozen tissue. This product is used for identifying the basic neuronal structure in brain or spinal cord sections.

Myelinated Fibers: Blue
Nissl Substance: Violet
Nerve Cells: Violet

Control Tissue

Tissues fixed in 10% formalin are suitable for use prior to paraffin embedding. Consult references (Kiernan, 1981; Sheehan & Hrapchak, 1980) for further details on specimen preparation.

1. Cut sections, usually 3 to 5 μm and pick the sections up on glass slides.
2. Bake the slides for at least 30 minutes at approximately 70°C.
3. Allow to cool.

Recommended Positive Control

1. Cerebral Cortex
2. Spinal Cord

Reagents Provided

Kit Contents	Volume	Storage
Cresyl Echt Violet Solution	125 mL	2-8°C
Luxol Fast Blue Solution	125 mL	15-30°C
Lithium Carbonate Solution (0.05%)	500 mL	15-30°C
Alcohol, Reagent (70%)	500 mL	15-30°C

Storage and Handling

Do not use product after the expiration date printed on vial. If reagents are stored under conditions other than those specified here, they must be verified by the user. Diluted reagents should be used promptly.

Staining Procedure

1. Deparaffinize sections if necessary and hydrate to distilled water.
2. Incubate slide in Luxol Fast Blue Solution for 24 hours at room temperature or 2 hours at 60°C.
3. Rinse thoroughly in distilled water.
4. Differentiate section by dipping in Lithium Carbonate Solution (0.05%) several times (up to 20 seconds).
5. Continue differentiation by repeatedly dipping in Alcohol, Reagent (70%) until gray-matter is colorless and white-matter remains blue.
6. Rinse slide in distilled water.
7. Incubate slide in Cresyl Echt Violet (0.1%) for 2-5 minutes.

8. Rinse quickly in 1 change of distilled water.
9. Dehydrate quickly in 3 changes of absolute alcohol.
10. Clear as desired and mount in synthetic resin.

Limitations of the Procedure

1. Histological staining is a multiple step diagnostic process that requires specialized training in the selection of the appropriate reagents, tissue selections, fixation, processing, preparation of the slide, and interpretation of the staining results.
2. Tissue staining is dependent on the handling and processing of the tissue prior to staining.
3. Improper fixation, freezing, thawing, washing, drying, heating, sectioning, or contamination with other tissues or fluids may produce artifacts or false negative results.
4. The clinical interpretation of any positive staining, or its absence, must be evaluated within the context of clinical history, morphology and other histopathological criteria. It is the responsibility of a qualified pathologist to be familiar with the special stain and methods used to produce the slide.
5. Staining must be performed in a certified licensed laboratory under the supervision of a pathologist who is responsible for reviewing the stained slides and assuring the adequacy of positive and negative controls.

Precautions

1. Consult local and/or state authorities with regard to recommended method of disposal.
2. Materials of human or animal origin should be handled as biohazardous materials and disposed of with proper precautions.
3. Avoid microbial contamination of reagents. Contamination could produce erroneous results.
4. This reagent may cause irritation. Avoid contact with eyes and mucous membranes.
5. If reagent contacts these areas, rinse with copious amounts of water.
6. Do not ingest or inhale any reagents.

Troubleshooting

If unexpected staining is observed which cannot be explained by variations in laboratory procedures and a problem is suspected, contact Diagnostic BioSystems Technical Support at (925) 484-3350, extension 2 or techsupport@dbiosys.com.

References

- I. Sheenan, D.C., Hrapchak, B.B. Theory and Practice of Histotechnology, 2nd Edition. Battelle Press, Columbus, OH. Page 262-264. 1980.
- II. Clark, G., et al., Staining Procedures. 4th Edition. Williams & Wilkins. Pages 146-147. 1981.
- III. Kluver, H., Barrera, E.A. A Method for the combined staining of cells and fibers in the nervous system. Journal of Neuropathology and Experimental Neurology, 1953, 12: pages 400-403.

