

KLUVER-BARRERA METHOD FOR MYELIN AND NERVE FIBERS

PURPOSE:	For In Vitro Diagnostic Use: Intended for the qualitative demonstration of myelin.
PRINCIPLE:	Luxol Fast Blue binds to the lipoproteins in myelin by salt bridges. Excess dye is removed with an Alcohol Solution. Cresyl Violet stains the nuclei.
CONTROL:	Brain, spinal cord <i>Control Slides can be purchased from Histology Control Systems. See inside back cover, Item# cs022.</i>
SPECIMEN PREPARATION:	Formalin fixed, paraffin embedded sections cut at 15 micrometers
SOLUTIONS:	1. Luxol Fast Blue 0.1% Alcoholic Item# s235 2. Cresyl Echt Violet 0.1% Aqueous Item# s167C 3. Lithium Carbonate 0.05% Aqueous Item# s2048 4. Reagent Alcohol 70% Item# c818D <i>Solutions can be purchased separately from Poly Scientific.</i>
NOTES:	
REFERENCE:	Kluver, H. and Barrera, E. <u>J. Neuropath. Exp. Neurol.</u> 12: 400-403, 1953.

STAINING PROCEDURE:

1. Deparaffinize in 2 changes each of Xylene, Reagent Alcohol, and Reagent Alcohol 95% (drain off excess 95% Alcohol).
2. Luxol Fast Blue 0.1% Alcoholic at 56–60°C overnight.
3. Rinse in Reagent Alcohol 95% to remove excess stain.
4. Rinse in distilled water.
5. Begin differentiation by quick immersion in Lithium Carbonate 0.05% Aqueous.
6. Continue differentiation in Reagent Alcohol 70% until gray and white matter can be distinguished.
7. Wash in distilled water.
8. Finish differentiation by rinsing briefly in Lithium Carbonate 0.05% Aqueous then putting through several changes of Reagent Alcohol 70% until the greenish blue of the white matter contrasts sharply with the colorless gray matter.
9. Rinse thoroughly in distilled water.
10. Cresyl Echt Violet 0.1% Aqueous for 6 minutes.
Note: Just before use add 15 drops of 10% Glacial Acetic Acid for each 100 mL. Filter and then preheat to 57°C.
11. Differentiate in several changes of Reagent Alcohol 95%.
12. Dehydrate in Absolute Alcohol and clear in Xylene, 2 changes each.
13. Mount with Poly Mount (Item# s2153) or any other acceptable mounting medium.

RESULTS:

Myelin Blue
Cell Products Including Nissal Fibers..... Pink to Violet

Poly Scientific R&D Corp.

Revision: B-18

