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Histology Special Stain - Cresyl Violet - Royal Oak

Document Type: Procedure

I. PURPOSE AND OBJECTIVE:

The purpose of this document is to provide a procedure for the demonstration of Nissl substance, which is rough endoplasmic reticulum found in the cell body and dendrites of nerve cells. This stain can be used to identify nerves in tissue sections, or the demonstration of chromatolysis, which is the loss of Nissl substance.

II. PRINCIPLE:

The reaction uses a basic aniline dye to stain the RNA found in the rough endoplasmic reticulum. By varying the time for differentiation, nuclei and Nissl substances can be demonstrated or only Nissl substances.

III. SPECIMEN COLLECTION AND HANDLING:

A. Fixation
    1. Any well-fixed tissue.
    2. 10% neutral buffered formalin preferred.
    3. Avoid mercuric fixatives

B. Processing
    1. Standard processing.

C. Section Thickness
    1. Routine specimens-8-10µm.

D. Slide Drying

1. 60 minutes at 60°C.

E. Type of slide

1. Plain slides

IV. REAGENTS:

A. **Cresyl Violet**

Cresyl Violet Acetate	**0.50 g**
Sodium acetate	**0.18 g**
Distilled water	**500.00 mL**
Acetic acid, concentrated	**1.50 mL**

1. Dissolve cresyl violet acetate and sodium acetate in distilled water.
2. Slowly add acetic acid, drop by drop, to solution.
3. Should have a pH of 3.5.
    a. If solution pH is below 3.5, add more sodium acetate.
    b. If solution pH is above 3.5, add more acetic acid.
4. Filter.
5. Let stand overnight before using.
6. Store at room temperature.
7. Stable for months.
8. May be reused until weak.

V. EQUIPMENT:

A. Balance

VI. SUPPLIES:

A. Erlenmeyer flasks
B. Graduated cylinders
C. Acid clean coplin jars
D. Funnel
E. Filter paper

VII. QUALITY CONTROL:

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

VIII. SPECIAL SAFETY PRECAUTIONS:

A. Cresyl Violet
    1. Is an irritant.

B. Sodium Acetate
    1. Has low hazard for recommended handling.

C. Acetic Acid
    1. Add drop by drop to solution.
    2. May cause eye and skin burns.

IX. PROCEDURE:

Step	Action	Time	Notes
1	Deparaffinize and hydrate slides through graded alcohol to distilled water.		
2	Place sections in cresyl violet solution.	**1-2 hours room temperature or 6 minutes in 60°C oven**	
3	Differentiate in two changes of 95% ethanol until nuclei and Nissl granules remain violet and the background is nearly colorless.		Check differentiation with the microscope. 1-2 drops of acetic acid may be added to the first alcohol to speed up differentiation.
4	Dehydrate through graded alcohols.		
5	Clear in two changes of xylene.		
6	Coverslip.		

X. LIMITATIONS:

A. Use the microscope to differentiate.
    1. Differentiation may need to be repeated several times.

B. To demonstrate only Nissl substance, continue to differentiate until the nuclei are colorless.

XI. RESULTS:

A. Nissle granules - **violet**

B. Nuclei - **violet**

C. Bacteria, fungus - **blue to purple**

D. Cartilage, mast cell granules - **blue to purple**

E. Background - **colorless**

XII. REFERENCES:

A. Carson FL: Histotechnology: A Self-Instructional Text, Chicago, IL, ASCP Press, 1990.

B. 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