

Beaumont

Origination 11/2/2022
Last 11/2/2022
Approved
Effective 11/2/2022
Last Revised 11/2/2022
Next Review 11/1/2024

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Applicability Royal Oak

Histology Special Stain - Toluidine Blue pH 2.9 Amyloid Stain - Royal Oak

Document Type: Procedure

I. PURPOSE AND OBJECTIVE:

The purpose of this document is to provide a procedure for the demonstration of amyloid in tissue sections.

II. PRINCIPLE:

Amyloid will stain metachromatically with toluidine blue; they will stain a different color from the dye solution and the rest of the tissue. The control section should show amyloid stained blue-purple and the background lighter blue. The color shift, called metachromasia, is generally attributed to the cationic or basic dye and is somewhat dependent on pH, dye concentration, and temperature.

III. SPECIMEN COLLECTION AND HANDLING:

- A. Fixation
 - 1. Any well-fixed tissue
- B. Processing
 - 1. Standard processing
- C. Section Thickness
 - 1. Routine specimens 5µm
- D. Slide Drying

1. 60 minutes at 60°C
- E. Type of slide
1. Plain

IV. REAGENTS:

A. **Toluidine Blue**

Toluidine blue O	1.0 gm
Isopropyl alcohol	50.0 mL
Distilled water	50.0 mL

Dissolve together . Adjust pH to 2.9 with HCL. Store at room temperature. Stable for months. May be reused until weak.

V. EQUIPMENT:

- A. Balance
- B. 37°C oven
- C. Magnetic stirrer

VI. SUPPLIES:

- A. Erlenmeyer flasks
- B. Graduated cylinders
- C. Coplin jars
- D. Forceps

VII. QUALITY CONTROL:

- A. Use a section of tissue with amyloid as a positive control.
- B. The pH of the toluidine blue solution must be at 2.9.

VIII. SPECIAL SAFETY PRECAUTIONS:

- A. Toluidine Blue O
 1. Is an irritant.
- B. Isopropyl Alcohol
 1. Flammable liquid and vapor.
- C. Hydrochloric Acid
 1. May cause burns.

IX. PROCEDURE:

Step	Action	Time	Notes
1	Deparaffinize and hydrate slides through graded alcohol to distilled water.		
2	Place in toluidine blue solution in a 37°C oven.	30 minutes	
3	Carefully blot section with filter paper.		
4	Immerse in isopropyl alcohol.	1 minute	
5	Carefully blot section with filter paper.		
6	Clear in 2 changes of xylene.		
7	Coverslip.		

X. RESULTS:

- A. Amyloid - **blue-violet**
- B. Background - **lighter blue**
- C. Amyloid with fluorescence microscope - **orange-red**

XI. LIMITATIONS:

Toluidine blue is extracted from sections by water and ethanol. Isopropanol extracts much less dye from sections than other dehydrating agents.

XII. REFERENCES:

- A. Carson FL: Histotechnology: A Self-Instructional Text, Chicago, IL, ASCP Press, 1990.
- B. Vacca LL: Laboratory Manual of Histochemistry. New York, NY, Raven Press, 1985

Approval Signatures

Step Description	Approver	Date
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Applicability

Royal Oak

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