

Beaumont

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Melanin Bleach

Document Type: Procedure

I. PURPOSE:

The purpose of this document is to provide a procedure to decolorized melanin in tissue to visualize cytologic details.

II. PRINCIPLE:

Identification of melanin may be made on the basis of generally characteristics of the substance and certain tests. Melanin is insoluble in organic solvents and weak acids and bases; however, it dissolves in strong alkali. Melanin is bleached by oxidizing agents. One of these agents is acidified potassium permanganate.

III. SPECIMEN COLLECTION AND HANDLING:

A. Fixation

1. Any well fixed tissue, 10% neutral buffered formalin preferred

B. Processing

1. Standard, overnight processing.

C. Section Thickness

1. Cut paraffin sections at 5 um.

D. Slide Drying

1. Dry slides for 30 minutes at 60⁰C.

E. **Type of slides**

1. Use charged slides, since sections tend to fall off during the treatment process.

IV. REAGENTS:

A. **1% POTASSIUM PERMANGANATE**

Potassium permanganate	1.0 gm
Distilled water	100.0 mL

Dissolve together. Store at room temperature; stable for months.

B. **WORKING POTASSIUM PERMANGANATE**

1% Potassium Permanganate	2.0 mL
Distilled water	28.0 mL

Mix together. Use for one day and discard.

C. **2% OXALIC ACID**

Oxalic acid	2.0 gm
Distilled Water	100.0 mL

Dissolve together. Store at room temperature; stable for several months.

D. **WORKING OXALIC ACID**

2% Oxalic Acid	6.0 mL
Distilled water	38.0 mL

Mix together. Use for one day and discard.

V. EQUIPMENT:

- A. Balance
- B. Magnetic stirrer

VI. SUPPLIES:

- A. Erlenmeyer flasks
- B. graduated cylinders
- C. force

VII. QUALITY CONTROL (QC):

- A. Run two duplicate controls- one bleached and one not bleached to serve as control.
- B. Section of tissue with melanin.

VIII. SPECIAL SAFETY PRECAUTIONS:

A. **POTASSIUM PERMANGANATE**

1. may cause skin and eye irritation. Harmful if inhaled.

B. **OXALIC ACID**

1. is a strong reducer. Contact with other material may cause fire. Store separate from other chemicals. May cause skin and eye burns. Irritating to respiratory tract.

IX. PROCEDURE

Step	Action	Time	Notes
1	Deparaffinize and hydrate sections through graded alcohol to distilled water.		
2	If necessary, remove mercury deposits with Lugol's		
3	Place slides in working Potassium Permanganate solution	1-6 hours	
4	Decolorize slides in Working Oxalic Acid		
5	Check under microscope, and repeat steps 3 and 4 if all of the melanin has not been bleached	1-2 minutes	
6	Wash in running water	10 seconds	
7	Continue with desired stain.		Omit bluing sections after staining with Hematoxylin, as the sections have a tendency to become detached from the slide.

X. RESULTS

- A. Melanin- Decolorized

XI. LIMITATIONS:

- A. Use charged slides, since sections tend to fall off during process

XII. REFERENCES:

1. Bancroft JD, Stevens A: Theory and Practice of Histological Techniques, 3rd ed. New York, NY, Churchill Livingstone, 1990.
2. Carson FL: Histotechnology: A Self-Instructional Text, Chicago, IL, ASCP Press, 1990.
3. Sheehan DC, Hrapchak BB: Theory and Practice of Histotechnology, 2nd edition. Columbus, Ohio, Battelle Press, 1980.
4. Histopathology Laboratory, Armed Forces Institute of Pathology, Washington, D.C. 20306

Approval Signatures

Step Description	Approver	Date
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Applicability

Royal Oak

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