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Beaumont

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Area	Laboratory-Histology
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Histology Special Stain - Esterase - Royal Oak

Document Type: Procedure

I. PURPOSE AND OBJECTIVE:

The purpose of this document is to provide a procedure for the demonstration of non-specific esterases. Esterases are enzymes that hydrolyze esters of carboxylic acids. Non-specific esterases can be found in liver, kidney, intestine, endoplasmic reticulum mitochondria, and lysosomes. This method demonstrates neutrophils and mast cells, which is important in hematologic diagnosis.

II. PRINCIPLE:

The reaction is an azo-dye reaction. Esterase is an enzyme found in lysosomes. It is a hydrolase, meaning it adds or removes water. It hydrolyzes carboxylic acids. The dye used is pararosaniline, to which sodium nitrite is added. Sodium nitrite adds azo groups (-N=N-) to the pararosaniline, which function as chromophores, making a deeper color. The acetate part of the naphthol AS-D chloracetate is the substrate. The esterase in the tissue will react with the acetate, releasing the naphthol compound. Naphthol compound will react with the azotized pararosaniline, making an insoluble azo dye. Disodium phosphate dibasic and potassium phosphate monobasic are the buffer, at pH 7.6.

Naphthol-AS-D-Chloracetate + Esterase (tissue) → Naphthol-AS-D

Naphthol-AS-D + Azotized Pararosaniline → red azo dye

III. SPECIMEN COLLECTION AND HANDLING:

A. Fixation

1. Any well-fixed tissue.
2. 10% neutral buffered formalin preferred.
3. Mercuric fixatives may also be used.

B. Processing

1. Standard, overnight processing.

C. Section Thickness

1. Routine specimens 4-5um.

D. Slide Drying

1. 60 minutes at 60°C.

E. Type of Slide

1. Plain slides

IV. REAGENTS:

A. **4% Sodium Nitrite (Solution A)**

Sodium nitrite	**0.4 g (in oxidizer cabinet)**
Distilled water	**10.0 mL**

JUST BEFORE USE, dissolve together.

B. **4% NEW FUCHSIN (SOLUTION B) (in freezer)**

New fuchsin	**1.0 g**
Distilled water	**20.0 mL**
Concentrated HCL	**5.0 mL**

Dissolve together. Place in 5 mL cuvettes, in 1.0 mL aliquots. Store in freezer. JUST BEFORE USE remove one aliquot from freezer and allow to warm up to room temperature.

C. **Substrate (Solution D)**

Naphthol AS-D Chloracetate	**0.002 g (in freezer)**
N,N-dimethylformamide	**1.0 mL** (in flammable cabinet)

JUST BEFORE USE, dissolve together.

D. **Sodium Phosphate Dibasic Buffer, M/15**

Sodium Phosphate Dibasic	**9.5 g**
Distilled water	**1000.0 mL**

Dissolve together. Store in refrigerator (3°C); stable for 3-6 months.

E. **Potassium Phosphate Monobasic, M/15**

Potassium phosphate monobasic	**9.1 g**
Distilled water	**1000.0 mL**

Dissolve together. Store in refrigerator (3^{o} C.); Stable for 3-6 months.

F. **Buffer**

Sodium phosphate dibasic, M/15	**17.4 mL (in fridge)**
Potassium phosphate monobasic, M/15	**2.6 mL (in fridge)**

JUST BEFORE USE, mix together. Only 19.0 mL are needed, and 20.0 mL were made, DISCARD 1.0 mL.

G. **Hexazotize Fuchsin**

4% Sodium nitrite (Solution A)	**1.0 mL (in muscle cabinet)**
4% Basic fuchsin (Solution B)	**1.0 mL**

JUST BEFORE USE, combine together. Swirl together, until well mixed. Allow to set on counter

for 1 to 3 minutes before proceeding with next step.

H. **Incubating Medium**

Buffer	**19.0 mL**
Hexazotize Fuchsin	**0.1 mL (3 drops from pipet)**
Substrate (Solution D)	**01.0 mL**

JUST BEFORE USE, add hexazotize fuchsin to buffer. Swirl together.
Pour this mixture into the substrate (Solution D). Combine in order just described. (solution turns pink)

I. **Hematoxylin**
Use Mayer or Gill hematoxylin from H&E set-up

J. **Dilute Ammonia Water**
Use dilute ammonia water from H&E set-up.

V. EQUIPMENT:

A. Mettler balance

B. 37°C oven

VI. SUPPLIES:

A. Erlenmeyer flasks

B. Graduated cylinders

C. Pipettes

VII. QUALITY CONTROL:

Section of bone marrow.

VIII. SPECIAL SAFETY PRECAUTIONS:

A. Sodium Nitrite

1. Is an oxidizing metal.
2. Store separately from all other reagents.

B. New Fuchsin

1. Is an irritant and a suspected carcinogen.

C. Naphthol As-D Chloracetate

1. Not a hazardous substance or mixture.

D. N,N-Dimethylformamide

1. May cause liver and kidney damage.
2. Harmful if inhaled, absorbed through skin, or swallowed.
3. May be irritating to skin and eyes.

4. Is a combustible liquid and vapor.

E. Sodium Phosphate Dibasic
    1. Has low hazard for recommended handling.

F. Potassium Phosphate Monobasic
    1. Has low hazard for recommended handling.

G. Hematoxylin
    1. Is incompatible with oxidizers and alkalies.
    2. Store separately from them.

H. Ammonium Hydroxide
    1. May cause severe skin and eye burns.
    2. Vapors are irritating to eyes and respiratory tract.
    3. Harmful if swallowed or inhaled.

I. Hydrochloric Acid
    1. Causes severe skin and eye burns.
    2. Harmful if inhaled or swallowed.

IX. PROCEDURE:

Step	Action	Time	Notes
1	Deparaffinize and hydrate slides through graded alcohol to distilled water.		
2	Lay slides flat in a lid of a staining dish.		
3	Pour incubating solution over slides.		Cover to prevent evaporation. Incubating solution should appear a light to medium pink when made.
4	Incubate slides at room temperature.	10 minutes	
5	Wash slides in running tap water.	5 minutes	
6	Stain slides in Mayer or Gill hematoxylin.	2 minutes	
7	Wash slides in running tap water.	5 minutes	
8	Blue in dilute ammonia water.	2-3 seconds	
9	Wash slides in running tap water.	5-10 minutes	

10	Remove slides from distilled water, and place upright on a paper towel to drain. Allow to air dry completely.		
11	Place slides in 2-3 changes xylene.	5-10 seconds each	
12	Coverslip using a synthetic mounting media.		

X. LIMITATIONS:

A. The following may influence the validity of test results:

1. Do NOT Lugolize slides. Placing slides through iodine and sodium thiosulfate will result in a false negative reaction.
2. Acetates of the naphthol AS series are hydrolyzed slowly; therefore, sites with low enzyme activity may not show up. Various types of esterases exhibit different affinities for the naphthol AS derivatives.
3. When making up the incubating solution, it is critical to mix the reagents in the order given.
4. Azotized solution must set for 1 to 3 minutes, before proceeding with other steps.

XI. RESULTS:

A. Neutrophil granules - **red**

B. Mast cell granules - **red**

C. Nuclei - **blue**

XII. REFERENCES:

A. Bancroft JD, Stevens A: Theory and Practice of Histological Techniques, 3rd ed. New York, NY, Churchill Livingstone, 1990.

B. Sheehan DC, Hrapchak BB: Theory and Practice of Histotechnology, 2nd edition. Columbus, Ohio, Battelle Press, 1980.

C. Vacca LL: Laboratory Manual of Histochemistry. Raven Press. 1985.

Approval Signatures

Step Description	Approver	Date
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Applicability

Royal Oak