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Histology Muscle Enzyme - Alkaline Phosphatase - Royal Oak

Document Type: Procedure

I. PURPOSE AND OBJECTIVE:

The purpose of this document is to provide a procedure for the demonstration of muscles that are undergoing degeneration. Alkaline phosphatase is a hydrolytic enzyme found in lysosomes. It is non-specific and hydrolyzes a wide range of phosphate esters. It will also demonstrate a positive reaction in connective tissue in a disease process called polymyositis. Macrophages and blood vessel walls will also demonstrate positive staining. In normal muscles, positive staining is found only in blood vessel walls. Muscle fibers undergoing regeneration also gives a diffuse staining.

II. PRINCIPLE:

a-Napthyl Phosphate is the substrate used in this protocol which is hydrolyzed and then coupled to the diazonium salt (Fast Blue B) which is then precipitated at the site of enzyme activity.

III. SPECIMEN COLLECTION AND HANDLING:

A. Fixation
    1. Unfixed tissue that has been frozen.

B. Processing
    1. Fresh tissue.
    2. No processing.

C. Section Thickness

1. Cut frozen sections at 8-12µ.

D. Slide Drying

1. None.

E. Type of Slide

1. Plus slides.

IV. REAGENTS:

A. **0.2 M Borate Buffer**

Boric Acid	**6.18 gm**
Distilled Water	**200.00 ml**

pH to 8.8 with NaOH and bring final volume to 500mL with distilled water. Store in refrigerator; good for one year.

B. **M Magnesium Sulfate**

Magnesium Sulfate	**1.2 gm**
Distilled Water	**100.0 ml**

Dissolve together. Store at room temperature; good for one year.

C. **Incubating Solution**

a-Napthyl Phosphate	**0.01 gm**
Fast Blue B Salt	**0.01 gm**
0.1 M MgSO4	**2.00 ml**
0.2 M Borate Buffer	**18.00 ml**

Make fresh; discard after use.

D. **10% Neutral Buffered Formalin**

E. **Aqueous Mounting Media**

V. EQUIPMENT:

A. Mettler balance

B. 37°C oven

VI. SUPPLIES:

A. Erlenmeyer flasks

B. Graduated cylinders

C. Funnel

D. Coplin jars

E. Pipets

F. Filter paper

VII. SPECIAL SAFETY PRECAUTIONS:

Follow standard safety procedures when preparing stains.

VIII. QUALITY CONTROL:

Frozen section of muscle with blood vessels.

IX. LIMITATIONS:

pH of incubation solution must be a pH in the alkaline range (pH 8.74).

X. PROCEDURE:

Step	Action	Time	Notes
1	Place slides in incubation solution and place into in a 37°C oven.	1 hour	
2	Rinse quickly in running tap water.		
3	Post fix in 10% formalin.	10 minutes	
4	Wash in running water.	15 minutes	
5	Rinse in distilled water.		
6	Mount using an aqueous mounting media.		Apathy gum syrup works well as the aqueous mounting medium.

XI. RESULTS:

Sites of alkaline phosphatase activity - **black, granular deposits**

XII. REFERENCES:

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

B. Histochemical Archives of Neurology, 1971.

Approval Signatures

Step Description	Approver	Date

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Applicability

Royal Oak