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Histology Muscle Enzyme - No Fade Myophosphorylase - Royal Oak

Document Type: Procedure

I. PURPOSE AND OBJECTIVE:

The purpose of this document is to provide the procedure for a no fade phosphorylase stain.

II. PRINCIPLE:

Myophosphorylase staining is used to diagnose McArdle's disease. The enzyme phosphorylase is essential in the breakdown of glycogen which is usually found in the liver and skeletal muscle. The absence of phosphorylase leads to an excess amount of glycogen storage. In this staining method, the phosphorylase in the tissue will react with glucose-2-phosphate and glycogen creating amylose. This amylose will then form various lengths of polysaccharide chains which are subsequently stained with iodine. In the absence of phosphorylase, the resulting stain will be non-existent.

III. DEFINITIONS:

A. **Irritant** – a biological, chemical, or physical agent that stimulates a characteristic function or elicits a response, especially an inflammatory response.

B. **Corrosive** – a chemical that causes obvious damage to living tissue or metals.

IV. SPECIMEN COLLECTION AND HANDLING:

A. Fixation

1. Cold acetone.

B. Processing
    1. Frozen section.

C. Section Thickness
    1. Routine specimens - 10µ.

D. Slide Drying
    1. None.

E. Type of slide
    1. Charged.

V. EQUIPMENT:

A. 60°C oven
B. pH meter

VI. SUPPLIES:

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

VII. SPECIAL SAFETY PRECAUTIONS:

A. Hydrochloric Acid
    1. Is corrosive.

B. Adenosine-5'-Monophosphate
    1. Is an irritant.

C. Potassium Iodide
    1. Is an irritant.

D. Iodine
    1. Is corrosive.

E. Acetone
    1. Is an irritant

VIII. REAGENTS:

A. **Acetone**

Cold acetone	**40.0 mL**

Chill in ice bath for a minimum of 10 minutes before use.

B. **1 M Sodium Acetate**

Sodium Acetate	**13.6 gm**
Distilled water	**1000.0 mL**

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

C. **1 M Sodium Acetate Buffer, pH 5.4**

1 M Sodium Acetate	**100.0 mL**
1 N Hydrochloric Acid	**17.0 gm**
Distilled Water	**385.0 mL**

Slowly add 1 N hydrochloric acid to solution. Stir together. pH to 5.4 by adding more hydrochloric acid or more sodium acetate. Store in refrigerator or room temperature; stable for months.

D. **Incubating Medium (make solutions just before use)**

Glycogen (shellfish or oyster)	**0.007 gm**
1 M Sodium Acetate Buffer	**10.0 mL**

Dissolve glycogen in sodium acetate buffer then add:

Glucose-1-Phosphate, potassium salt	**0.05 gm**
Distilled Water	**5.0 mL**

Dissolve glucose in distilled water and add to glycogen solution, then add:

Adenosine-5'-Monophosphate	**0.01 gm**
Distilled Water	**5.0 mL**

Dissolve adenosine in distilled water and add to glycogen/glucose solution.

E. **40% Alcohol**

100% alcohol	**40.0 mL**
Distilled Water	**60.0 mL**

Combine alcohol and water. Store at room temperature; stable for months.

F. **100% Alcohol**

G. **Gram's Iodine**

Potassium Iodide	**2.0 gm**
Distilled Water	**20.0 mL**
Iodine	**1.0 gm**
Distilled Water	**280.0 mL**

Dissolve the potassium iodide in the 20 mL of distilled water then add the remaining reagents. Store at room temperature; stable for one year. Can also use vendor pre-made Gram's iodine solution.

IX. QUALITY CONTROL(QC):

Frozen section of muscle.

X. LIMITATIONS:

Shellfish/Oyster glycogen seems to work the best.

XI. PROCEDURE:

Step	Action	Time	Note
1	Fix slides in cold acetone.	5 minutes	Acetone should be cooled in ice bath for a minimum of 10 minutes prior to use.
2	Pour incubating solution over slides.	1 hour	Make solution just before use. Cover to prevent evaporation. Incubate in 37°C oven.
3	Rinse in two changes of distilled water.		
4	Place in 40% alcohol.	2 minutes	
5	Place in 100% alcohol.	2 minutes	
6	Place in Gram's Iodine.	6 minutes	
7	Dehydrate and clear.		
8	Coverslip.		

XII. RESULTS:

A. Normal Staining Fibers – **red to brown to blue** (dependent on polysaccharide unit lengths)

B. Phosphorylase Negative – **no staining at all**

XIII. REFERENCES:

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

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

Approval Signatures

Step Description	Approver	Date
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Applicability

Royal Oak