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Histology Muscle Enzyme - Phosphorylase Stain - Royal Oak

Document Type: Procedure

I. PURPOSE AND OBJECTIVE:

The purpose of this document is to provide a procedure for the demonstration of amylophosphorylase, an enzyme that breaks down glycogen. It is found in high concentration in the liver, and in skeletal and cardiac muscles. Type II muscles have more phosphorylase than Type I muscle, but this stain should not be used for fiber typing. The absence of this enzyme would indicate the glycogen storage disease known as McArdles's disease.

II. PRINCIPLE:

A. The reaction has the phosphorylase in the tissue reacting with the substrate glucose-1-phosphate and glycogen, forming amylose, which is further formed into units of polysaccharides of varying lengths. Iodine causes a color formation, which is dependent upon the length of the polysaccharide. The ethanol inhibits the formation of branching units of polysaccharides. The AMP is an energy source.

Glucose-1-Phosphate + Phosphorylase (tissue) → Amylose
Amylose + Phosphorylase (Tissue) → Polysaccharide
Polysaccharide + Iodine → Color

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 10µ.

D. Slide Drying

1. None.

E. Type of slide

1. Plus slides.

IV. REAGENTS:

A. **1 M Sodium Acetate**

Sodium Acetate	**82.03 gm**
Distilled water	**1000.0 mL**

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

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

1 M Sodium Acetate	**100.0 mL**
1 N hydrochloric acid	**17.0 mL**
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 (4°C.) or room temperature; stable for months.

C. **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, and then add:

Glucose-1-phosphate potassium salt (dissolve 0.05 gm in 5 mL distilled water) 5.0 mL

D. **Adenosine-5'-monophosphate (AMP)**

(Dissolve .01gm in 5 mL distilled water).	**5.0 mL**
Absolute Alcohol	**4.0 mL**

Adjust the pH to 5.8-6.0 with 1 N hydrochloric acid or 1 M Sodium Acetate Buffer.

E. **Weigert Iodine**

Potassium iodide	**2.0 gm**
Iodine crystals	**1.0 gm**
Distilled water	**100.0 mL**

Completely dissolve potassium iodide in 10 mL distilled water. Add iodine crystals, and stir until completely dissolved. Add the other 90 mL distilled water. Store in dark bottle at room temperature; stable for months.

F. **Dilute Weigert Iodine**

Weigert Iodine	**25.0 mL**

Distilled water	**25.0 mL**

G. **Iodine-Glycerol Mounting Medium**

Weigert iodine	**2.0 mL**
Glycerol	**20.0 mL**

Mix together. Store in refrigerator (4^{O} C.); stable for months; warm to room temperature before use.

V. EQUIPMENT:

A. Metler balance
B. 60°C oven
C. pH meter

VI. SUPPLIES:

A. Erlenmeyer flasks
B. Graduated cylinders
C. Funnel
D. Coplin jars
E. Pipets

VII. QUALITY CONTROL(QC):

Frozen section of muscle.

VIII. SPECIAL SAFETY PRECAUTIONS:

A. Hydrochloric Acid
    1. Is an acid.
    2. Add slowly, drop by drop, to solution.
    3. May cause severe skin and eye burns.
B. Sodium Acetate
    1. Is an irritant.
C. Glucose-1-Phosphate (Potassium Salt)
    1. No MSDS sheets.
D. Adenosine-5'-Monophosphate (AMP)
    1. No MSDS sheets.
    2. Store in refrigerator.
E. Potassium Iodide

1. Is an irritant.

F. Iodine

1. Is a corrosive and an oxidizer, may be irritating to eyes and respiratory tract; can cause skin and eye burns.

IX. PROCEDURE:

Step	Action	Time	Notes
1	Pour incubating solution over slides.	1 hour	Make solution just before use. Cover to prevent evaporation. Incubate in 37°C. oven
2	Rinse in 2 changes of distilled water.		
3	Transfer to diluted Weigert Iodine	10 seconds	
4	Wet mount using iodine-glycerol mounting medium.		DO NOT DEHYDRATE, CLEAR OR USE PERMANENT MOUNTING MEDIA. When somewhat dry, seal edges of coverslip with nail polish. Read results IMMEDIATELY AFTER COVERSLIPPING as the stain begins to bleed into the mounting media quickly.

X. LIMITATIONS:

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

1. Shellfish/Oyster glycogen seems to work the best.

XI. RESULTS:

Intermyofibrillar network of sarcoplasm - **slate gray to blue**
(Type II fibers are more intense than type I)

XII. REFERENCES:

A. Stanford Medical Center Neuropathology lab

B. Sarnat, Harvey, B. Muscle Pathology and Histochemistry, 1984: pgs. 139-140

Approval Signatures

Step Description	Approver	Date

Medical Director	Kurt Bernacki: System Med Dir, Surgical Path	1/18/2023
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Applicability

Royal Oak