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Histology Muscle Enzyme - Acid Phosphatase - Royal Oak

Document Type: Procedure

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I. PURPOSE AND OBJECTIVE:

The purpose of this document is to provide a procedure for the demonstration of muscle fibers that have been denervated and are undergoing degeneration. Acid phosphatase is a hydrolytic enzyme found in lysosomes. It is non-specific and catalyzes a wide range of phosphate esters. An acid maltase deficiency will give a positive reaction. In normal muscle, possibly very small amount might be found in the muscle fibers and the connective tissues.

II. PRINCIPLE:

The reaction is an azo-dye reaction. 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. Naphthol AS-B1 supplies the phosphate as the substrate. The acid phosphatase in the tissue will react with the phosphate, releasing the naphthol compound. The naphthol compound will react with the azotixed pararosaniline, making an insoluble azo dye. Veronal acetate is the buffer. Sodium hydroxide is used to pH the substrate to pH between 4.7 and 5.0.

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. **Veronal Acetate Buffer**

Sodium Acetate Trihydrate (M.W. 136)	**1.94 gm**
Sodium Barbiturate (M.W. 206)	**2.94 gm**
Distilled water	**100.0 ml**

Dissolve together. Store in refrigerator (4°C.); stable for months.

B. **Pararosaniline Stock Solution**

Pararosaniline (basic fuchsin)	**4.0 gm**
Distilled water	**80.0 ml**
Hydrochloric Acid, concentrated (HCL)	**20.0 ml**

Stir pararosaniline (basic fuchsin) into water. Slowly add hydrochloric acid, drop by drop, to the above solution. Warm solution gently to 65°C., until pararosaniline is completed dissolved. Cool to room temperature. Filter. Store in refrigerator (4°C.); stable for months.

C. **4% Sodium Nitrite**

Sodium nitrite	**4.0 gm**
Distilled water	**100.0 ml**

Dissolve together. Store in refrigerator (4°C.); stable 2-4 months.

D. **Naphthol AS-B1 Phosphate Substrate**

Naphthol AS-B1 Phosphate	**0.01 gm**
Dimethyl Formamide	**1.0 ml**

Dissolve together. MAKE FRESH JUST BEFORE USE.

E. **Azotized Pararosaniline**

Pararosaniline Stock	**0.8 ml**
4% Sodium Nitrite	**0.8 ml**

Mix together JUST BEFORE USE. Swirl solutions together. Allow to set on counter 1-2 minutes before proceeding with next step.

F. **0.1 N Sodium Hydroxide**

Sodium hydroxide	**0.4 gm**
Distilled water	**100.0 ml**

Add sodium hydroxide to water slowly. Mix together; stable at room temperature for months.

G. **Incubating Solution**

Azotized pararosaniline	**1.6 ml**
Naphthol AS-B1 Phosphate substrate	**1.0 ml**
Veronal Acetate Buffer	**5.0 ml**

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Distilled water	**13.0 ml**

MIX TOGETHER JUST BEFORE USE. Adjust pH to between 4.7 to 5.0 with 0.1 N sodium hydroxide. Filter before use.

H. **0.2% Light Green**

Light Green SF Yellowish	**0.2 gm**
Distilled water	**98.8 ml**
Acetic acid	**0.2 ml**

Mix together. Store at room temperature; stable for months; may be reused until weak.

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

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VII. SPECIAL SAFETY PRECAUTIONS:

A. Sodium Acetate, Trihydrate

1. Is an irritant.

B. Sodium Barbiturate

1. Toxic; harmful if swallowed or inhaled.

C. Pararosaniline (Basic Fuchsin)

1. Is an irritant and a suspected carcinogen.

D. Hydrochloric Acid

1. Is an acid.
2. Slowly add drop-by-drop to solution.
3. May cause severe eye and skin burns.

E. Sodium Nitrite

1. Is an oxidizing metal.
2. Harmful if inhaled or swallowed.

F. Naphthol AS-B1 Phosphate

1. Not a hazardous substance.

G. Dimethylformamide
    1. Is toxic.
    2. Can cause liver and kidney damage.
    3. Harmful if inhaled, absorbed through skin, or swallowed.
    4. Can cause eye and skin irritation.

H. Sodium Hydroxide
    1. Is a corrosive.
    2. May cause severe skin and eye burns; harmful if inhaled.

I. Light Green SF Yellowish
    1. This chemical is non-hazardous.

J. Acetic Acid
    1. Is an acid.
    2. Add drop by drop to solution.
    3. May cause skin or eye burns.

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VIII. QUALITY CONTROL:

A. Frozen section of muscle with positive area for acid phosphatase.
B. Frozen section of prostate or liver.

IX. LIMITATIONS:

A. Azotized pararosaniline must set on counter 2 to 3 minutes to allow the azo groups to bind. Failure to do so will result in a negative to decreased reaction.
B. Make up the substrate solution, sodium nitrite and incubating solution just before use.
C. Do not take stain through alcohols or positively stained activity will be lost.
D. Sodium nitrate can be made up just before use.

X. PROCEDURE:

Step	Action	Time	Notes
1	Pour incubation solution over slides.		Cover to prevent evaporation.
2	Incubate in 37°C oven.	1 hour	pH of incubating solution must be between 4.7 –5.0.
3	Rinse in distilled water, 3 changes.	5-10 seconds	
4	Counterstain with 0.2% light green.	10-15	

		seconds	
5	Rinse quickly in distilled water, 2-3 changes	5-10 seconds	
6	Blot and air-dry sections.		
7	Dip in xylene and coverslip using a synthetic mounting media.	5 seconds	

XI. RESULTS:

A. Abnormal muscle fibers (degenerating) - **red**

B. Macrophage - **red**

C. Sites of acid phosphatase activity - **red**

D. Muscle fibers, normal - **green**

XII. REFERENCES:

A. Bancroft JD and Steven 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, Ohio, Battelle Press, 1980.

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Approval Signatures

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Applicability

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