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Histology Muscle Enzyme - Esterase - Royal Oak

Document Type: Procedure

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. Nerves and motor end plates will also be stained. Type I muscle fibers will stain more darkly than Type II, but this stain should not be used to determine fiber typing.

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. Acetate 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 is the buffer. Hydrochloric acid or sodium hydroxide is used to pH the substrate to pH 6.3.

Naphthol-AS-Acetate + Esterase (tissue) → Naphthol-AS

Naphthol-AS + Azotized Pararosaniline → orange to red/brown azo-dye

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. **0.2 M Phosphate Buffer**

Sodium Phosphate Dibasic (Na_2HPO_4	**10.35 gm**
Distilled water	**500.00 ml**

Dissolve together. Adjust pt pH 7.4 with 1N HCl. Stable at room temperature or in refrigerator 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°., until pararosaniline is completed dissolved. Cool to room temperature. Filter. Store in refrigerator; 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. **1% Alpha-Naphthyl-AS-Acetate**

Alpha-Naphthyl-AS-Acetate	**1.0 gm**
Acetone	**100.0 ml**

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

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. **1 N Hydrochloric Acid**

Hydrochloric acid, concentrated	**37.0 ml**
Distilled water	**963.0 ml**

Slowly add hydrochloric acid to water. Stable at room temperature for months.

G. **1 N Sodium Hydroxide**

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

Slowly add sodium hydroxide to water. Stir together; stable at room temperature for months.

H. **Incubating Solution**

0.2 M Phosphate buffer	**20.0 ml**
1% Alpha-Naphthol-AS-Acetate	**0.5 ml**
Azotized pararosaniline	**1.6 ml**

MIX TOGETHER JUST BEFORE USE. MIX TOGETHER IN THE ORDER GIVEN. Adjust pH to 6.3 using either 1 N hydrochloric acid or 1 N sodium hydroxide. Solution should be yellow-red.

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:

A. **Sodium Phosphate Dibasic**

1. **Has low hazard for recommended handling.**

B. **Pararosaniline (Basic Fuchsin)**

1. **Is an irritant and a suspected carcinogen.**

C. **Hydrochloric Acid**

1. **Is an acid.**
2. **Add slowly, drop-by-drop, to solution.**
3. **May cause skin and eye burns.**

D. **Sodium Nitrite**

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

E. **Alpha-Naphthol-AS-Acetate**

1. **No material safety data sheet (MSDS) sheet.**

F. **Acetone**

1. **Is an extremely flammable liquid and vapor.**
2. **Vapor may cause flash fire.**

G. **Sodium Hydroxide**

1. **Causes severe eye and skin burns.**
2. **Harmful if inhaled.**

VIII. QUALITY CONTROL:

Frozen section of muscle with nerve.

IX. LIMITATIONS:

A. **Use 30% hydrogen peroxide, and dilute JUST BEFORE USE.**

B. **If incubating solution appears reddish immediately before or after pH, discard.**

C. **pH of incubating solution must be pH 6.3.**

D. **pH of the incubating solution is critical. If pH drifts to the alkaline (pH 6.8 or above) nothing other than a faint yellow staining of the whole section will occur. A shift to the acid (pH 5.9 and below) will give an orange-red non-specific staining.**

E. **When making up the incubating solution, it is critical to mix the reagents in the order given.**

X. PROCEDURE:

Step	Action	Time	Notes
1	Place slides in incubating solution.	1 hour	Make Just Before Use. Cover and incubate in a 37°C oven. After incubation, if solution is a dark yellow-orange with clumps of reddish precipitate, this is a good indication that the stain is working properly.
2	Wash slides in running tap water.	10 minutes	
3	Dehydrate through graded alcohols, clear with xylene.		
4	Coverslip using a synthetic mounting media.		

XI. RESULTS:

A. **Abnormal muscle fibers - dark-orange to red**

B. **Nerves - red to reddish brown**

C. **Type I muscle may appear darker than Type II muscle**

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.**

Approval Signatures

Step Description	Approver	Date
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Applicability

Royal Oak