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Histology Muscle Enzyme - Cytochrome Oxidase Stain - Royal Oak

Document Type: Procedure

I. PURPOSE AND OBJECTIVE:

The purpose of this document is to provide a procedure for the demonstration of a type of oxidoreductase enzyme known as cytochrome oxidase. This oxidative enzyme catalyzes the reaction between the substrate and oxygen. It is found in the mitochondria. Absence of this enzyme is associated with a progressive muscle weakness that is usually fatal in childhood. This stain can be used for muscle typing, Type I stains darker the Type II, as it has more mitochondria. This stain can also be used to indicate architectural changes in the muscle, such as swirls, target cells, and central core disease, all of which have a displacement of the mitochondria. It will also show depletion or clumping of the mitochondria.

II. PRINCIPLE:

The reaction is an oxidation-reduction reaction. Hydrogen peroxide is the substrate. The cytochrome oxidase enzymes in the muscle will split the hydrogen peroxide into water and oxygen. The oxygen will oxidize the dye, which is 3,3'-diaminobenzidine (DAB). This forms a brown precipitate at the sites of enzyme activity. Sodium acetate is the buffer. Manganese chloride is an activator. This reaction will only happen in the presence of manganese.

$$\text{Hydrogen peroxide } (H_2O_2) + \text{cytochrome oxidase (tissue)} \rightarrow H_2O + O_2$$

$$O_2 + \text{DAB} \rightarrow \text{oxidized DAB} \rightarrow \text{brown precipitate}$$

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. **0.1 M Sodium Acetate Buffer, Ph 5.6**

Sodium acetate, trihydrate	**6.804 gm**
Distilled water	**500.0 ml**

Dissolve together. Adjust pH to 5.6 with acetic acid. Store in refrigerator; stable for months.

B. **1% Manganese Chloride**

Manganese chloride	**1.0 gm**
Distilled water	**100.0 ml**

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

C. **0.1% Hydrogen Peroxide**

3% hydrogen peroxide	**0.5 ml**
Distilled water	**14.5 ml**

Mix together JUST BEFORE USE.

D. **1% Cupric Sulfate**

Cupric sulfate	**1.0 gm**
Distilled water	**100.0 ml**

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

E. **1N Sodium Hydroxide**

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

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

F. **Incubating Medium**

DAB (3,3'-diaminobenzidine tetrahydrochloride	**0.03 gm**
M sodium acetate buffer	**13.5 ml**

1% manganese chloride	**1.5 ml**
0.1% hydrogen peroxide	**0.15 ml**

Mix together JUST BEFORE USE. Adjust pH to 5.5 with 1 N sodium hydroxide or concentrated acetic acid. Filter before use.

V. EQUIPMENT:

A. Metler 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 Acetate

1. Is an irritant.

B. Acetic Acid

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

C. Manganese Chloride

1. Is an irritant.
2. Is toxic.

D. Hydrogen Peroxide

1. Is an oxidizer.
2. Contact with other material may cause fire.
3. Store in refrigerator.
4. Can cause severe eye burns and skin burns.
5. Harmful if inhaled or swallowed.
6. Vapor is irritating to eyes and respiratory system.

E. Cupric Sulfate

1. Is an irritant.

F. DAB (3,3'-diaminobenzidine tetrahydrochloride)
    1. Is a suspected carcinogen.
    2. Wear gloves when preparing and using the incubating medium.

G. Sodium Hydroxide
    1. is corrosive and may cause severe eye and skin burns.

VIII. QUALITY CONTROL(QC):

Frozen section of muscle. (Built-in control, as all tissue has mitochondria).

IX. LIMITATIONS:

Use 3% hydrogen peroxide, and dilute JUST BEFORE USE

X. PROCEDURE:

Step	Action	Time	Notes
1	Place slides in incubating medium solution.	1 hour	Make Just Before Use. Cover and incubate in a 37°C. oven. Incubating solution will be light brown in color. Tissue will appear light brown after incubation.
2	Rinse in distilled water, 2-3 changes.	5-10 seconds	
3	Place in 1% cupric sulfate.	5 minutes	
4	Rinse in distilled water, 2-3 changes.	30 seconds total	
5	Dehydrate through graded alcohols, clear with xylene.		
6	Coverslip using a synthetic mounting media.		

XI. RESULTS:

A. Mitochondria - **brown**
B. Type I fibers - **darker brown**
C. Type II fibers - **lighter brown**
D. Myofibrils - **unstained**

XII. REFERENCES:

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

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