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Histology Muscle Enzyme - ATPase - Royal Oak

Document Type: Procedure

I. PURPOSE AND OBJECTIVE:

The purpose of this document is to provide a procedure for the demonstration of ATPase (adenosine triphosphatase). ATPase enzymes are responsible for the degradation of adenosine triphosphate (ATP), which causes a release in energy. The muscle ATPase is involved in muscle contraction. By varying the pH, type Ia, IIb and IIc muscle fibers can be differentiated. The size and shape of individual fibers and the distribution of fiber types is determined with this stain. Changes in the size, shape, or checkerboard distribution occur with denervation or muscular dystrophies.

II. PRINCIPLE:

The ATPase enzyme in the tissue hydrolyses the substrate adenosine triphosphate (ATP). This produces adenosine diphosphate (ADP) and a phosphate group. This phosphate group reacts with the calcium chloride, forming calcium phosphate, which is a colorless precipitate. When combined with the cobalt chloride, the cobalt is exchanged for the calcium, forming cobalt phosphate, which is also colorless. When treated with ammonium sulfide, cobalt sulfide is formed, which is a black precipitate. Calcium chloride is used in the incubating medium to activate the myosin ATPase and inhibit the mitochondrial ATPase. By pre-incubation at various pH's, the different myosin ATPase can be distinguished. Pre-incubation of tissue sections at a high pH (9.3 to 10.4) extracts low activity myosin ATPase from the muscle fibers. Pre-incubation at a low pH (4.2 – 4.3) extracts high activity myosin ATPase from the muscle fibers. Sodium barbital and sodium acetates are buffers. Sodium hydroxide, hydrochloric acid, and acetic acid are used to adjust the pH of the buffers.

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. **01. M Sodium Barbital Solution**

Sodium barbital	**10.0 gm**
Distilled water	**500.0 ml**

Dissolve together. Store in refrigerator; stable for months.

B. **0.18 M Calcium Chloride Solution**

Calcium chloride	**9.9 gm**
Distilled water	**500.0 ml**

Dissolve together. Store in refrigerator; stable for months.

C. **N Sodium Hydroxide**

Sodium hydroxide	**4.0 gm**
Distilled water	**1000.0 ml**

Slowly and carefully add sodium hydroxide to water, taking care not to splash. Stir together until dissolved. Store at room temperature; stable for months.

D. **N Hydrochloric Acid**

Hydrochloric acid	**3.7 ml**
Distilled water	**996.3 ml**

Slowly add hydrochloric acid, drop by drop, to water. Mix together. Store at room temperature; stable for months.

E. **Sodium Acetate Solution**

Sodium acetate	**13.0 gm**
Distilled water	**500.0 m**

Dissolve together. Store in refrigerator; stable for months.

F. **Acetic Acid Solution**

Acetic acid, concentrated	**6.0 ml**
Distilled water	**494.0 ml**

Slowly add acetic acid, drop by drop, to distilled water. Mix together. Store in refrigerator; stable for months.

G. **Calcium Chloride Rinse**

Calcium chloride	**5.0 gm**
Distilled water	**500.0 ml**

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

H. **Cobalt Chloride Rinse**

Cobalt chloride	**10.0 gm**
Distilled water	**500.0 ml**

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

I. **1% Ammonium Sulfide**

Ammonium sulfide	**1.0 ml**
Distilled water	**99.0 ml**

Mix together JUST BEFORE USE. Make solution in fume hood. Wear gloves. Mix together. All solutions should be discarded with lots of running water.

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. biSodium Bartal

1. Irritant to skin, eyes, respiratory tract.
2. Harmful if swallowed.

B. Calcium Chloride

1. Is an irritant to eyes, skin and respiratory tract.

C. Sodium Hydroxide

1. Is a corrosive.

2. Can cause severe eye and skin burns.

D. Hydrochloric ACID
    1. Is an acid.
    2. Add slowly, drop by drop to water.
    3. Can cause severe eye and skin burns.
    4. Can cause lung damage.
    5. Harmful if swallowed.

E. Acetic Acid
    1. Is an acid.
    2. Add slowly, a drop at a time, to solution.
    3. Can cause severe eye and skin burns.

F. Cobalt Chloride
    1. Is harmful if inhaled or swallowed.
    2. Can cause skin and eye irritation.

G. Ammonium Sulfide
    1. Is a corrosive and a flammable liquid.
    2. Can cause eye and skin burns.
    3. Keep away from open flames.
    4. Obnoxious odor.
    5. Use only under hood.

VIII. QUALITY CONTROL:

Frozen section of muscle. (Built-in control, as all muscle has myosin ATPase).

IX. LIMITATIONS:

A. While rinsing in the various solutions after incubation, be certain to shake the jar, get the solutions behind the slides, and that the slides do not stick together. Failure to remove all the previous solutions will result in a black precipitate that cannot be removed.

B. With time, this stain will fade. It may no longer be possible to differentiate muscle types on slides that have been stored for months to years.

C. pH of all solutions MUST be exact.

X. PREPARATIONS:

A. **Incubating Medium, Ph 9.8**

0.1 M Sodium barbital solution	**30.0 ml**

0.18 M Calcium chloride solution **15.0 ml**
Distilled water **105.0 ml**
ATP (adenosine triphosphate) **0.225 gm**

JUST BEFORE USE, mix together in the order given. Adjust pH to 9.8 with 0.1 N sodium hydroxide. Filter into three coplin jars.
Cover coplin jars with lids, and place solution in 37°C oven to warm up, while making the rest of the solutions.

B. **Calcium-Barbital Buffer, Ph 9.8**

Calcium chloride **0.444 gm (0.222gm)**
Sodium barbital **0.412 gm (0.206 gm)**
Distilled water **100.0 ml (50.0 mL)**

JUST BEFORE USE, dissolve together. Adjust pH to 9.8 using either 0.1 N hydrochloric acid or 0.1 N sodium hydroxide.

C. **Acetate Buffer, Ph 4.3**

Sodium acetate solution **15.0 ml**
Acetic acid solution **40.0 ml**

JUST BEFORE USE, mix together. Adjust pH to 4.3 using wither more sodium acetate or acetic acid solution.

XI. PROCEDURE:

Step	Action	Time	Notes
1	Place slides labeled pH 9.8 into calcium barbital buffer, ph 9.8.	15 minutes	Make Just Before Use. See preparation below.
2	Place slides labeled pH 4.3 into acetate buffer, pH 4.3.	5 minutes	Make Just Before Use. See preparation below.
3	Place each set of slides directly into 2 separate jars of incubating medium.	20 minutes	Make Just Before Use. See preparation below. Incubate in 37°C oven. Place slides from pre-incubating solutions DIRECTLY into incubating solution.
4	Rinse all slides in calcium chloride rinse, 2 changes.	10 seconds each	
5	Rinse all slides in cobalt chloride rinse, 1 change.	10 seconds	Slides can be combined at this point. This will save on amount of reagent used.
6	Place all slides in another cobalt chloride rinse.	3 minutes	
7	Rinse in distilled water, 2-4 changes.	5-10 seconds	
8	Place all slides in 1% ammonium sulfide.	3 minutes	Tissues will appear colorless until the ammonium sulfide is applied. Use ammonium sulfide under hood, as the odor is obnoxious, and the fumes are injurious.
9	Rinse all slides in distilled water,	10	

	2-4 changes.	seconds each	
10	Dehydrate through graded alcohols, clear with xylene.		
11	Coverslip using a synthetic mounting media.		

XII. RESULTS:

ENZYME	I	II		
		IIA	IIB	IIC
ATPase, pH 9.8	**light**	**dark**	**dark**	**dark**
ATPase, pH 4.6	**dark**	**light**	**medium**	**dark**

XIII. 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