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Histology Muscle Enzyme - NADH - 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 enzyme known as dehydrogenases using NADH (Nicotinamide Adenine Dinucleotide, reduced). These are oxidative enzymes that remove a hydrogen ion from a substance. It is found in the mitochondria and is involved in the breakdown of glucose. This stain can be used for muscle typing, as Type I stains darker than 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. Carcinoma of the colon and malignant polyps will also stain.

II. PRINCIPLE:

The reaction is an oxidation-reduction reaction. NADH is the substrate. The dehydrogenase enzymes in the muscle will remove the hydrogen from the NADH (= oxidation). This hydrogen then reduces the tetrazolium salt, nitro-blue tetrazolium (NBT). This forms a highly colored formazan dye which is finely granular blue.

NADH + dehydrogenase (tissue) → NAD^- + H^+

H^+ + Nitro blue tetrazolium (NBT) → reduced tetrazolium → formazan (blue)

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 Stock Solutions**

Solution A: Sodium phosphate, monobasic	**13.8 gm**
Distilled water	**500.0 mL**
Solution B: Sodium phosphate, dibasic	**2.8 gm**
Distilled water	**500.0 mL**

B. **0.2 M Phosphate Buffer Working Solution**

Solution A	**54.0 ml**
Solution B	**146.0 ml**

C. pH to 7.2. Store in refrigerator; stable for months.

D. **Ringer's solution**

Sodium chloride	**7.00 gm**
Calcium chloride	**0.30 gm**
Potassium chloride	**0.25 gm**
Distilled water	**1000.00 ml**

Store in refrigerator; stable for months.

E. **0.2% p-Nitro Blue Tetrazolium (NBT)**

NBT	**0.2 gm (0.024 gm)**
Distilled water	**100.0 mL (12.0 mL)**

Make just before use.

F. **Incubating solution**

NADH	**0.01 gm**
Distilled water	**6.80 ml**
0.2% NBT	**12.00 ml**
0.2M Phosphate buffer	**4.80 ml**
Ringer's solution	**3.80 ml**

pH to 7.2. Make just before use.

V. EQUIPMENT:

A. Mettler balance

B. 37°C oven

C. pH meter

VI. SUPPLIES:

A. Erlenmeyer flasks

B. Graduated cylinders

C. Funnel

D. Coplin jars

E. Pipets

VII. SPECIAL SAFETY PRECAUTIONS:

Follow standard safety procedures when preparing stains.

VIII. QUALITY CONTROL (QC):

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

IX. PROCEDURE:

Step	Action	Time	Notes
1	Pour incubating solution over slides.	1 hour	Make Just Before Use. Allow buffer to come to room temperature before use. Cover and incubate in a 37º oven.
2	Rinse with rinse buffer, several changes.	5-10 seconds each	
3	Rinse with 30% Acetone, 60% Acetone, 30% Acetone.	10 seconds each	
4	Dehydrate through graded alcohols, clear in xylene.		
5	Coverslip using a synthetic mounting media.		

X. RESULTS:

Sites of enzyme activity - **purple deposits**

XI. REFERENCES:

A. Stanford Medical Center Neuropathology lab, Stanford CA - Santa Clara Valley Medical Center Neuropathology lab, San Jose

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