

# Beaumont

Origination 1/18/2023  
 Last 1/18/2023  
 Approved  
 Effective 1/18/2023  
 Last Revised 1/18/2023  
 Next Review 1/17/2025

Document Sharon Scalise:  
 Contact Supv, Laboratory  
 Area Laboratory-  
 Histology  
 Applicability Royal Oak

## Histology Muscle Enzyme - Succinic Dehydrogenase Stain - Royal Oak

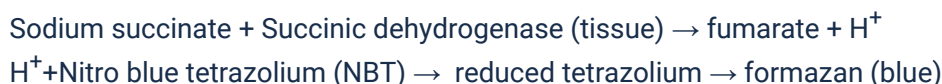
Document Type: Procedure

### I. PURPOSE AND OBJECTIVE:

The purpose of this document is to provide a procedure for the demonstration of succinic dehydrogenase. Succinic dehydrogenase is an anaerobic oxidative enzyme that removes a hydrogen ion from a substrate. It is found in the mitochondria and is involved in the Krebs cycle. 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. Red-ragged fibers, which contain an accumulation of bizarre mitochondria around the rim of each fiber, are especially evident with this stain. Denervated muscles stain dark.

### II. PRINCIPLE:

- A. The reaction is an oxidation-reduction reaction. Sodium succinate is the substrate. The succinic dehydrogenase enzymes in the muscle will remove a hydrogen from the sodium succinate (=oxidation). This hydrogen then reduces the tetrazolium salt, nitro-blue tetrazolium (NBT). This forms a highly colored formazan dye which is finely granular blue. Sodium phosphate and potassium phosphate are used as 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 10 $\mu$ .
- D. Storage
  - 1. Store slides in refrigerator.
- E. Type of slide
  - 1. Plus slides.

### IV. REAGENTS:

- A. **0.2 M Sodium Succinate**

<b>Sodium Succinate</b>	<b>8.1 gm</b>
<b>Distilled water</b>	<b>250.0 ml</b>

Mix together. Store in brown bottle in refrigerator; expires in 1 year.
- B. **0.2 Phosphate Buffer**

<b>Sodium phosphate Dibasic, Anhydrous</b>	<b>11.36 gm</b>
<b>Potassium Phosphate Monobasic</b>	<b>2.7 gm</b>
<b>Distilled water</b>	<b>500.0 ml</b>

Mix together well. Store in brown bottle in refrigerator; expires in 1 year.
- C. **SDH Incubating Solution**

<b>0.2 M Sodium Succinate</b>	<b>10.0 ml</b>
<b>0.2 M Phosphate buffer</b>	<b>10.0 ml</b>
<b>Nitro Blue Tetrazolium (NBT)</b>	<b>0.02 gm</b>

JUST BEFORE USE, mix together. Adjust pH to 7.2 – 7.6.

### V. EQUIPMENT:

- A. Mettler balance
- B. 60°C oven
- C. pH meter

### VI. SUPPLIES:

- A. Erlenmeyer flasks

- B. Graduated cylinders
- C. Funnel
- D. Coplin jars
- E. Pipets

## VII. SPECIAL SAFETY PRECAUTIONS:

- A. Sodium Succinate (Succinic acid)
  - 1. Is an irritant.
- B. Nitro Blue Tetrazolium
  - 1. Is an irritant.
- C. 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. PROCEDURE:

Step	Action	Time	Notes
1	Pour SDH incubating solution over slides using a plastic mailer.	2 hours (or longer)	Make solution just before use. Warm buffer to room temperature before use. Cover to prevent evaporation. Incubate in 37°C. oven. Tissues will appear blue after incubation
2	Rinse in distilled water.	1 minute	
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. LIMITATIONS:

- A. Store sodium succinate and NBT in freezer until use.
- B. Tissue must be unfixed, as SDH is very sensitive to fixation.
- C. Not all the NBT will dissolve at room temperature. It will finish dissolving during the 37°C. incubation.

D. Autolysis does not immediately damage enzyme activity. Autopsy material can be used.

## XI. RESULTS:

- A. Mitochondria - **blue**
- B. Type I fibers - **dark blue**
- C. Type II fibers - **light blue**
- D. Myofibrils - **unstained**
- E. Intermyoibrillar network (sarcoplasmic reticulum) - **unstained**

## XII. REFERENCES:

- A. California Society of Histotechnology, May 16, 1987. Diagnostic Muscle Biopsy Procedure, Aldana Martin.

### Approval Signatures

#### Step Description

#### Approver

#### Date

Medical Director

Kurt Bernacki: System Med Dir,  
Surgical Path

1/18/2023

Policy and Forms Steering  
Committee (if needed)

Sharon Scalise: Supv,  
Laboratory

1/17/2023

Policy and Forms Steering  
Committee (if needed)

Gail Juleff: Project Mgr Policy

1/17/2023

Amy Knaus: Dir, Lab Operations  
C

1/17/2023

Jennifer Lehmann: Mgr  
Laboratory

1/17/2023

Sharon Scalise: Supv,  
Laboratory

1/10/2023

### Applicability

Royal Oak