Status Active PolicyStat ID 12960807

Beaumont

Origination	1/18/2023	Document Contact	Sharon Scalise: Supv, Laboratory
Last Approved	1/18/2023	Area	Laboratory-Histology
Effective	1/18/2023	Applicability	Royal Oak
Last Revised	1/18/2023		
Next Review	1/17/2025		

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.

Sodium succinate + Succinic dehydrogenase (tissue) → fumarate + 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. Storage
    1. Store slides in refrigerator.

E. Type of slide
    1. Plus slides.

IV. REAGENTS:

A. **0.2 M Sodium Succinate**

Sodium Succinate	**8.1 gm**
Distilled water	**250.0 ml**

Mix together. Store in brown bottle in refrigerator; expires in 1 year.

B. **0.2 Phosphate Buffer**

Sodium phosphate Dibasic, Anhydrous	**11.36 gm**
Potassium Phosphate Monobasic	**2.7 gm**
Distilled water	**500.0 ml**

Mix together well. Store in brown bottle in refrigerator; expires in 1 year.

C. **SDH Incubating Solution**

0.2 M Sodium Succinate	**10.0 ml**
0.2 M Phosphate buffer	**10.0 ml**
Nitro Blue Tetrazolium (NBT)	**0.02 gm**

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. Intermyofibrillar 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
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Applicability

Royal Oak