Washington University School of Medicine
Neuromuscular Lab
CAP: 1923316
CLIA: 26D0652044
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SUCCINIC DEHYDROGENASE PROTOCOL

PRINCIPLE:

Succinic dehydrogenase (SDH), is a soluble iron flavoprotein that catalyzes the reversible oxidation of succinic acid to fumaric acid. The histochemical demonstration of the activity of this enzyme is achieved by incubation of fresh frozen sections with a succinate substrate in the presence of a tetrazolium compound. Tetrazoliums are water-soluble compounds employed in histochemistry as redox indicators. Under appropriate conditions, tetrazoliums are reduced to formazans which are water-insoluble colored compounds. Commonly used tetrazoliums include nitro blue tetrazolium (NBT). Enzymatic activity releases hydrogen from the substrate, and the released hydrogen is transferred to the tetrazolium. With the addition of hydrogen, the tetrazolium is converted to purple-blue formazan pigment marking the site of enzyme activity.

SPECIMEN REQUIRED:

Snap frozen human striated muscle. (Use the 2-methylbutane freezing method described elsewhere.)

Controls:

Specific external controls are not used by this laboratory for routine muscle histochemistry. There is always enzyme activity present in muscle. The degree and location is of pathological interest.

METHOD:

Fixation: None, use snap frozen tissue.

Technique: Cut 16 μm sections in cryostat from snap frozen biopsy. Attach one or more sections to a No. 1½, 22 mm square coverslip.

Equipment:

- ceramic staining rack - Thomas Scientific 8542e40 (used to store coverslips with sections before staining)
- columbia staining jar - Thomas Scientific 1229J30 (stain is performed in this jar)
- forceps
- latex gloves

Reagents:

- Acetone: Fisher A18 **FLAMMABLE**
- deionized water
- gelatin: Sigma G2500;
- Glycerol: Sigma G 5516, **IRRITANT**
- NBT (Nitrotetrazolium chloride): Goldbio #NBT1
- Phenol: Sigma P3946, **CAUSTIC**
- Sodium phosphate dibasic- BioXtra ≥ 99.0 (HNA_2O_4P): Sigma S7907

- Sodium phosphate monobasic monohydrate -ACS ≥ 98% (H_2NaO_4P – H20): Sigma 9638
- Sodium succinate dibasic hexa-hydrate- Reagent Plus ≥ 99.0 ($C_4H_4Na_2O_4$ – 6 H_20): Sigma S2378

Solutions:

I. **0.2 M Phosphate Buffer, pH 7.6** (combine13 ml 0.2 M sodium monobasic phosphate + 87 ml 0.2 M sodium dibasic phosphate):
store at 4^0C for up to two months

- 0.2 M sodium monobasic phosphate (NaH2PO4)
  27.8 gm/liter deionized H2O: (make fresh) 13 ml
- 0.2 M sodium dibasic phosphate (Na2HPO4) anhydrous
  stock: 28.39g/liter deionized water:
  (store at RT up to two months) 87 ml

II. **Staining Incubation medium;** *make fresh before use*

- 0.2 M phosphate buffer (solution 1) 10 ml
- Sodium Succinate: Sigma S2378 270 mg
- NBT: Goldbio #NBT1 10 mg

III. **Acetone Solutions; 30%, 60% and 90% ;** *make fresh before use*
Acetone in deionized water:

- 30%: 30 ml Acetone in 70 ml deionized water
- 60%: 60 ml acetone in 40 ml deionized water
- 90%: 90 ml acetone in 10 ml deionized water

IV. **Aqueous Mounting Medium (glycerol based):** *store at RT for up to one year.*

- Gelatin: Sigma G2500 4 g
- Glycerol: Sigma G 8773 25 ml
- phenol (**CAUSTIC**): Sigma P3946 0.5 ml
- deionized water 21 ml

1. Dissolve gelatin in boiling water.
2. Cool, but do not allow to solidify.
3. Add phenol and glycerol.
4. Mix well.
5. **Allow air bubbles in mixture to dissipate before using!**

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Staining Procedure:

1. Cut 16 µm sections in cryostat from snap frozen biopsy. Attach one or more sections to a No. 1½, 22 mm square coverslip.

2. Prepare Solution II: staining incubation medium.

3. Incubate coverslips in the staining incubation medium, using a columbia staining dish (Thomas Scientific 1229J30), overnight at room temperature.

4. Next day, wash with three exchanges of deionized H_2O.
   *Note: sections may be left in water overnight if it is desired.

5. Prepare Acetone solutions III: 30, 60 and 90 % acetone using deionized H_2O and remove unbound NBT from the sections with three exchanges each of the acetone solutions in the following order: 3 x 30%, 3 X 60%, then 3 X 90%. Leave the 90 % acetone covering the sections until a faint purplish cloud is seen over the section; ~ 30 seconds.

6. Finally, rinse 3 times with deionized H_2O and then mount the coverslips on slides with Solution IV: aqueous mounting medium.

7. Let coverslips dry.

Results:
Purple formazan precipitate is deposited at sites of mitochondria in sarcoplasmic network. Type I fibers are darker than those of type II. Results appear similar to those of the NADH stain but not as intense. Walls of blood vessels also are stained. Fibers that are designated as "ragged-red" by the Gomori trichrome stain, stain intensely dark with this stain. Best results occur if the sections are stained with the staining incubation medium on the same day that they are cut.

REFERENCES:

1. Sheehan, D.C. and Hrapchak, B.B.: THEORY AND PRACTICE OF HISTOTECHNOLOGY Second Edition, Battelle Memorial Institute,1987.