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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.

Controls:

Stain several different muscles simultaneously. There is normally always enzyme activity present in muscle. The degree and location is interpreted.

METHOD:

Fixation: None. Use snap frozen tissue.

Technique: Cut 10 - 16 micron (12 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 #8542-E40
Columbia staining dish - Thomas Scientific #8542-C12
Columbia staining dish(jar) - Thomas Scientific #8542-E30
Forceps Latex gloves

Reagents:

Acetone - Baxter #010-4 **FLAMMABLE**
deionized water
Gelatin - 100 bloom -ICN 960317 store at room temperature
Glycerol -Sigma G 8773, store at room temperature **IRRITANT**
Nitro blue tetrazolium - Sigma N6876 store desiccated at 0 - 5 ^{o} C
Phenol - Fisher A931-1, **CAUSTIC,** Store at room temperature
Sodium dibasic phosphate (Na_2HPO_4) anhydrous ACS
(FW 141.96)-Sigma S9763, Fisher S374, or Mallinckrodt 7917
Store at room temperature
Sodium dibasic phosphate (Na_2HPO_4) heptahydrate (FW 268.07)
Sigma S9390,or Fisher S373 , store at room temperature

Sodium monobasic phosphate (Na_2HPO_4)monohydrate

(FW 137.99) , ACS - Sigma 9638 **or** Fisher S369, or Mallinckrodt 7892, Store at room temperature

Succinic acid, disodium salt - Sigma S2378, Store at room temperature

Solutions:

I. 0.2 M Phosphate Buffer, pH 7.6

0.2 M sodium monobasic phosphate (Na_2HPO_4) (27.8 gm/liter deionized H_2O)	13 ml
0.2 M sodium dibasic phosphate (Na_2HPO_4) heptahydrate (53.65 gm/liter deionized H_2O) **OR** sodium dibasic phosphate (Na_2HPO_4) anhydrous (28.39g/liter deionized water)	87 ml

II. 0.2 M Succinic Acid (sodium salt) with deionized H_2O 5.4g/100ml

Prepare FRESH each time needed
(A stock solution may be kept refrigerated for two weeks and still be effective.)

III. Aqueous Mounting Medium (glycerogel)

Gelatin (ICN#960317 - 100 bloom	4 g
Glycerol	25 ml
Phenol (**CAUSTIC !**)	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!**

Staining Procedure:

1. Prepare the incubation medium as follows:

0.2 M phosphate buffer	10 ml
dissolve Sodium Succinate	270 mg
" NBT	10 mg

2. Incubate coverslips in a Columbia staining dish (Thomas Scientific #8542-C12) for overnight at room temperature.

3. Wash with three exchanges of tap or deionized H_2O.
   At this point, the sections may be left in water overnight if it desired.

4. Prepare approximate solutions of 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 increasing then decreasing concentration. Leave the 90 % acetone covering the sections until a faint purplish cloud is seen over the section.

5. Rinse several times with deionized H_2O and then mount the coverslips with the aqueous mounting medium.

Results:

Blue-Purple formazan precipitate is deposited at sites of mitochondria in sarcoplasmic network. Type I fibers are darker than those of type II. Fibers with mitochondrial proliferation, and small regenerating muscle fibers, stain intensely dark with this stain. Best results occur if the sections are stained 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.

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