

## **Succinate Dehydrogenase Stain -Fiber Typing in SKM**

modified from Neuromuscular Clinical Laboratory, Neurology, WUMS

### **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 succinic substrate in the presence of a tetrazolium compound. Tetrazolium is a water-soluble compound employed in histochemistry as a redox indicator. Under appropriate conditions, tetrazolium salts are reduced to formazans, which are water-insoluble colored compounds. Commonly used tetrazolium salts 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:**

Frozen muscle tissue should be cut in cross section. Use the isopentane freezing method described in the separate protocol.

### **Reagents:**

- Acetone: Baxter #014-4, store in flammable cabinet
- Gelatin: ICN #960317, 100 bloom, store at RT
- Glycerol: Sigma #G8773, store at RT
- Nitro Blue Tetrazolium: Sigma #N6876, dessicated at 4°C
- Phenol: Fisher A931-1, store at 4°C
- Sodium Dibasic Phosphate Heptahydrate ( $\text{Na}_2\text{HPO}_4$ ): Sigma #S9390, store at RT
- Sodium Monobasic Phosphate, ( $\text{NaH}_2\text{PO}_4$ ): Sigma #S9638, store at RT
- Succinic Acid, disodium salt: Sigma #S2378, store at RT

### **Solutions (make in advance):**

1. 0.2M Phosphate Buffer, pH 7.6

Make from these 2 stock solutions as follows:

0.2M Sodium Monobasic Phosphate (2.78 g/100ml)	13mls
0.2M Sodium Dibasic Phosphate Heptahydrate (26.8 g/500ml)	87mls

Store at RT, check pH before use.

2. Aqueous Mounting Medium (glycerogel)

Mix: Gelatin (100 bloom)	4 g
Glycerol	25 ml
Phenol	0.5 ml
ddH <sub>2</sub> O	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!

This should be made in advance. Before using, melt in a beaker of water on a hot plate at low heat until liquid. Store at RT.

3. Acetone Solutions:

Prepare approximate solutions of 30, 60, and 90% acetone using ddH<sub>2</sub>O. You will need about 400 mls of 30 and 60%, and about 200 mls of 90% per Coplin jar (up to 8 slides per jar). Store in Flammable cabinet.

**Staining Procedure:**

1. Prepare incubation medium as follows:

0.2M Phosphate Buffer	10 ml
Sodium Succinate	270 mg
NBT	10 mg

This requires about 400  $\mu$ l per slide.

2. Encircle section on slide with PAP Pen. Add ~400  $\mu$ l per slide and incubate for 30 minutes (mouse tissue) at 37°C. Note that it is difficult to walk to the incubator without spilling solution.
3. Transfer slides to Copling jar.
4. Wash slides with 3 exchanges of dH<sub>2</sub>O (or tap water).
5. Remove unbound NBT from the sections with 3 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.
6. Rinse several times with dH<sub>2</sub>O and then directly mount the coverslips (no dehydration steps) with the aqueous mounting medium.

**Results:**

Purple formazan precipitate is deposited at sites of mitochondria in sarcoplasmic network. Type I fibers are darker than those of type II. Walls of blood vessels are also stained. Best results occur if the sections are stained on the same day that they are cut.