

## Histochemical Stain for Cytochrome C Oxidase in Cultured Cells

1. Plate cells on sterile coverslips or in cell chamber slides 12-24 hours before staining.
2. Rinse in 3 changes of PBS with  $Mg^{++}$  and  $Ca^{++}$ .
3. Air dry
4. Incubate for 15 minutes at room temperature in preincubation medium:
  - 99.5mL Tris-Hcl 50mM pH 7.6
  - 10g sucrose
  - 28mg cobalt chloride
5. Rinse once in:
  - 0.1 M sodium phosphate pH 7.6
  - 10% sucrose
6. Incubate at 37°C for 2-6 hours in incubation medium:
  - 10 ml 10% sucrose in 0.1 M sodium phosphate pH 7.6
  - 10 mg cytochrome c Sigma C-7752
  - 10 mg DAB hydrochloride
  - 2.0 mg catalase Sigma C-40
  - Adjust to pH 7.4
  - Filter through 0.2 $\mu$ M syringe filter
7. Rinse once in:
  - 0.1 M sodium phosphate pH 7.6
  - 10% sucrose
8. Rinse once for 5 minutes in PBS
9. Rinse once for 5 minutes in distilled water
10. Mount with aqueous mounting medium.

## Histochemical Stain for Cytochrome C Oxidase in Muscle Cryostat Sections

1. Cut 8 $\mu$ m cryostat sections, pick up on coverslips and air dry
2. Incubate for one hour at 37°C in incubation medium:
  - 10mL Tris-Hcl 50mM pH 7.6
  - 10mg cytochrome c type IV
  - 50mg DAB hydrochloride
  - Adjust to pH 7.4 with 1N NaOH
3. Rinse 3x in distilled water
4. Post fix for 5 minutes at room temperature in 10% formalin
5. Rinse 3x in distilled water
6. Mount with aqueous mounting medium.

## Histochemical Stain for Succinate Dehydrogenase in Muscle Cryostat Sections

1. Cut 8µm cryostat sections, pick up on coverslips and air dry
2. Incubate for one hour at 37°C in incubation medium:
  - 10mL Tris-Hcl 50mM pH 7.6
  - 10mg Nitro Blue Tetrazolium
  - 20mg Succinic Acid Sigma S-7501
  - 0,25mg Phenazine Methosulphate
  - Adjust to pH 7.4 with 1N NaOH
3. Rinse 3x in distilled water
4. Mount with aqueous mounting medium.