

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 type IV (Sigma)
 - 10 mg DAB hydrochloride (Sigma)
 - 2.0 mg catalase (Sigma)
 - Adjust to pH 7.4
 - Filter through 0.2µM 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 Farrant's medium

Histochemical Stain for Cytochrome C Oxidase 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 cytochrome c type IV
 - 50mg DAB hydrochloride
 - Adjust to pH 7.4
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 Farrant's medium