Washington University School of Medicine
Neuromuscular Lab
CAP: 1923316
CLIA: 26D0652044
NY: PFI 3499

CYTOCHROME OXIDASE PROTOCOL

PRINCIPLE:

Cytochrome oxidase (COX) is the collective name for Complex IV of the oxidative respiratory chain of enzymes located in mitochondria. This method is a modification of the "Nadi" reaction. The use of 3,3' diaminobenzidine (DAB) results in a brown insoluble compound at the site of cytochrome oxidase 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:

Catalase (Sigma C-10)
Cytochrome C (Sigma C-2506
deionized water
3, 3' Diaminobenzidine tetrahydrochloride (Sigma D-5637)
CARCINOGENIC, store at -20^{o}C
Permount - Fisher SP15-100, **FLAMMABLE HEALTH HAZARD**
Reagent alcohol, ACS histochemical Fisher A962-4,or HPLC A995
FLAMMABLE, TOXIC, store at room temp. in flammable cabinet
Sodium dibasic phosphate (Na_2HPO_4) anhydrous ACS
(FW 141.96)-Sigma S9763, Fisher S374, or Mallinckrodt 7917
Store at room temperature
Sodium monobasic phosphate (Na_2HPO_4) monohydrate (FW 137.99)
Store at room temperature
Sucrose -Sigma S7903, Store at room temperature
Xylenes - Fisher #HC700-1GAL, **FLAMMABLE**,
Store at room temperature in flammable cabinet)

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Solutions:

I. 0.2 M Phosphate Buffer, pH 7.6 (at room temperature)
0.2 M sodium dibasic phosphate (Na_2HPO_4) anhydrous (28.39gm/liter ~95 ml
0.2 M sodium monobasic phosphate (Na_2HPO_4) Monohydrate
(27.8 gm/liter deionized H_2O) ~11 ml
Add the monobasic to the dibasic while adjusting
the final pH to ~7.6 at room temperature.

II. Incubating Solution:
7 mg DAB
12 mg cytochrome C
20 µg catalase (approximately a few crystals)
750 mg Sucrose
5 ml 0.2 M Phosphate Buffer
5 ml deionized H2O
Dissolve well.

III. Alcohol 50 % (v/v)
reagent alcohol ~50 ml
deionized water ~50 ml

IV. Alcohol 70 %(v/v)
reagent alcohol ~70 ml
deionized water ~30 ml

V. Alcohol 80 % (v/v)
reagent alcohol ~80 ml
deionized water ~20 ml

VI. Alcohol 95 % (v/v)
reagent alcohol ~95 ml
deionized water ~ 5 ml

Staining Procedure

1. Place coverslips in the incubating solution in a columbia staining dish (Thomas Scientific #8542-C12) for at least 1 hour but no more than 2 hours at 37°C or **overnight at room temperature**.

2. Wash with three exchanges of tap or deionized H_2O.

3. Dehydrate in ascending alcohols (50%,70%,80%,95% x 2, 100% x 2) in Columbia staining dish(jar) - Thomas Scientific #8542-E30.

4. Clear with at least 2 changes of xylene in Columbia staining dish - Thomas Scientific #8542-E30

5. Mount with PERMOUNT or another synthetic organic mounting medium.

Results

Sites of cytochrome oxidase activity are colored brown.

REFERENCES

1. Seligman et al., 1968. In: *HISTOCHEMISTRY - THEORETICAL and APPLIED,* A.G. Everson Pearse, Vol. 2, 3rd Edition; Williams & Wilkins, Baltimore, 1972.

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