



**VitroView™ COX/SDH Double Histochemical Stain Kit
(For 50-100 slides)**

SKU# VB-3022

Introduction

Observing the activity of respiratory enzymes is a straightforward approach for investigating mitochondrial dysfunction. Cytochrome c oxidase (COX) is essential for mitochondrial function. Succinate dehydrogenase (SDH) is entirely encoded by nuclear DNA and its activity is typically not affected by impaired mitochondrial DNA (mtDNA). Combining the COX with the SDH can demonstrate COX negative fibers. These may be SDH positive and may be ragged red fibers. Three of the 13 subunits of COX are encoded by mitochondrial DNA whereas SDH is encoded by nuclear DNA. Therefore SDH is not affected by mitochondrial DNA mutations. Ragged red fibers in mitochondrial myopathies are generally COX negative except in Mitochondrial Encephalopathy, Lactic acidosis, and Stroke-like episodes (MELAS). Intra fiber mosaicisms (mixture of bluish COX deficient and brownish COX positive mitochondria within the same fiber) are also well demonstrated by this combined technique. The impaired mtDNA observed in mitochondrial diseases, aging, and age-related diseases often leads to the presence of cells with low or absent COX activity.

Kit Components

SKU#	Reagent	Size
VB-3022-1	COX A Solution	1ml×5
VB-3022-2	COX B Solution	1ml×5
VB-3022-3	Succinate solution	0.3ml×5
VB-3022-4	Yellow SDH Incubation Medium	1.5 ml×5
VB-3022-5	COX Inhibitor Solution	1ml×2
VB-3022-6	SDH Inhibitor Solution	1ml×2

Storage

Store at -20°C

Method

1. Tissue preparation for cryosectioning
 - 1) Sacrifice the animal by either cervical dislocation or decapitation, in accordance with available ethical permit.
 - 2) Quickly collect tissues of interest without fixation, and rapidly freeze on dry ice (tissues may require freezing in isopentane) or propane chilled with liquid nitrogen to obtain optimal morphology).
 - 3) Store tissues in aluminum foil at -80 °C until ready to section.
 - 4) Embed frozen tissue in preparation for cryosectioning.
 - 5) Collect 10-14µm cryostat sections. Thaw sections onto slides at room temperature for 2-5 minutes, and store slides without cover-slipping at -20 °C until ready to use.

2. Prepare COX incubation solution: Defrost one COX A Solution (VB-3022-1) and one COX B Solution (VB-3022-2) vial. One vial of COX A solution are mixed with 1 vial of COX B solution.
3. Immediately put 80~200ul of COX incubation solution onto frozen sectioned slides in humidity chamber and incubate in dark for two hours at room temperature.
4. Check staining and replace for longer if required
5. Rinse slides in distilled water
6. Freshly prepare SDH incubation medium: Defrost a vial (0.3ml) of Succinate Solution (VB-3022-3) and a vial of Yellow SDH Incubation Medium (1.5 ml) (VB-3022-4); Add solution succinate solution to Yellow SDH Incubation Medium prior to use and mix well.
7. Add 80~200ul of SDH incubation medium onto slide and incubate at 37°C for 1-2 hour.
8. Rinse slides in distilled water.
9. Rinse in water, dehydrate, clear and mount.

Appropriate specificity controls

1. For specificity controls for COX activity, repeat "COX histochemistry" steps, and add 100µl of COX Inhibitor Solution (VB-2022-5), a terminal respiratory chain inhibitor.
2. For specificity controls for SDH activity, repeat "SDH histochemistry" steps with the removal of sodium succinate and the addition of 100µl of SDH Inhibitor Solution (VB-2022-6), a competitive inhibitor of SDH.

Results

Cytochrome Oxidase positive mitochondria-----	Brown
Cytochrome Oxidase negative mitochondria-----	Blue

References

1. Ross, J.M. Visualization of Mitochondrial Respiratory Function using Cytochrome C Oxidase / Succinate Dehydrogenase (COX/SDH) Double-labeling Histochemistry. *J. Vis. Exp.* (57), e3266, DOI : 10.3791/3266 (2011).
2. Seligman et al (1968) *J Cell Biol* 38:1-14.
3. Loughlin M. (1993). *Muscle biopsy. A laboratory investigation.* Butterworth-Heinemann p.38-39.
4. Sheehan D, Hrapchak B. (1987). *Histotechnology*, 2nd Ed. Batelle Press, Columbus p306-307

Note

This product is intended for research purposes only. This product is **not** intended to be used for therapeutic or diagnostic purposes in humans or animals.

Precautions

Handle with care in chemical hood. Avoid contact with eyes, skin and clothing. Do not ingest. Wear gloves.