SDH staining protocol. v1

This protocol is based on J. Histochem. (Nachals, NM et al. 5. 420-430, 1957) for staining skeletal muscle cross-section to determine if there is a change of mitochondrial SDH activity as an index of oxidative phenotype,

Procedure

1. Sacrifice mice by cervical dislocation under anesthesia followed by muscle sample harvesting. Cut the muscle across at the belly.
2. Freeze the muscle in a position that the muscle fibers are perpendicular to the bottom of the mold in OCT freezing compound (Tissue-Tek, Sakura) pre-chilled in liquid nitrogen. Wrap the tissue block in an aluminum foil and store in -80°C.
3. Cut skeletal muscle cross-sections (5 μm) using a cryostat and mount it on a positive charged glass slide, wrap in an aluminum foil and store at -80°C until use.
4. Place the slide with muscle section in a chamber, and incubate in prewarmed (37C°) Incubation Solution for 40 min at 37C° in a water bath. This incubation time can be extended to 60 min, if the staining is insufficient.
5. Wash the samples in distilled water (1 min x 3 times).
6. Wipe off water from slide glass (DO NOT touch muscle section), mount with Glycerol based mounting medium, and seal with nail polish.

Reagent

Incubation solution (20 ml)

Reagent	Source/Cat#	MW/Conc.	Quantity	Final Conc.
Sodium succinate	Sigma S2378	270.14/0.2 M	5 ml	50 mM
Phosphate buffer		0.2 M	5 ml	50 mM
Nitroblue tetrazoliumand	Sigma N5514	1 mg/ml	10 ml	0.5 mg/ml

Phosphate buffer stock solution (100 ml)

Reagent	Source/Cat#	MW/Conc.	Quantity	Final Conc.
KH2PO4	Fisher P380	136.09/1 M	12ml	0.12M
Na2HPO4	Fisher S374	141.96/1 M	88ml	0.88M

Glycerol based mounting medium (50 ml)

Reagent	Source/Cat#	MW/Conc.	Quantity	Final Conc.
Gelatine	Sigma G1890		2.5 g	5%
Glycerol	Fisher, G33-1		25 ml	50%
Distilled H2O			25ml	