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Version 1

TS Procure 812 - primary fixation Karnovsky's - transwells (TM - 013) V.1

DOI

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Protocol status: Working

We use this protocol and it's working

Created: May 13, 2023



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Protocol Integer ID: 81814

Keywords: Procure 812, formalin, epon, procure 812 resin block, transwells to procure, fixed transwell, resin block, ts procure, transwell, resin

Abstract

The method is used to Karnovsky's EM fixed transwells to Procure 812 resin blocks. The presence of 4% paraformaldehyde is only used to satisfy microsecurity requirements.

Guidelines

Sample is not optimal for EM, best to dissect and use only the outer 2mm of tissue mass. Sample is usually derived from histology.

Protocol materials

- ⊗ 25% Glutaraldehyde
- ⊗ 16% Paraformaldehyde ProSciTech Catalog #C004
- ⊗ Osmium Tetroxide ProSciTech
- ⊗ Ethanol
- ⊗ Propylene oxide ProSciTech Catalog #C156
- ⊗ Propylene oxide ProSciTech Catalog #C156
- ⊗ Propylene oxide ProSciTech Catalog #C156



HEADER

1 SAN:

SPEC No:

OPERATOR & STEPS:

OPERATOR & STEPS:

Conventional

5h 15m

- 2 Karnovsky's fixative: [M] 2.5 % volume 25% Glutaraldehyde and [M] 4 % volume 16% Paraformaldehyde ProSciTech Catalog #C004 in [M] 0.1 Mass Percent Sorenson's Phosphate buffer (pH 7.2, 300mosmol/kg) for at least 00:40:00 or overnight 40m
- 3 [M] 0.1 Mass Percent buffer 00:15:00 15m
- 4 [M] 1 % volume Osmium Tetroxide ProSciTech in buffer 01:00:00 1h
- 5 [M] 70 % volume Ethanol for at least 00:20:00 or overnight 20m
- 6 [M] 95 % volume Ethanol 00:20:00 20m



- 7 [IM] 100 % volume Ethanol 00:20:00 20m
- 8 [IM] 100 % volume Ethanol dried with molecular sieve 00:20:00 20m

Remove membrane

- 9 Remove membrane from transwell scaffold using a 45o scalpel and transfer to a glass vial with [IM] 100 % volume Ethanol dried with molecular sieve.

Conventional

5h 15m

- 10 [IM] 50 % volume Ethanol [IM] 50 % volume Propylene oxide **ProSciTech Catalog #C156** 00:20:00 20m
- 11 [IM] 100 % volume Propylene oxide **ProSciTech Catalog #C156** 00:20:00 20m
- 12 [IM] 50 % volume Propylene oxide **ProSciTech Catalog #C156**
[IM] 50 % volume Procure 812
- 13 [IM] 100 % volume Procure 812 00:20:00 20m
- 14 [IM] 100 % volume Procure 812 01:00:00 1h
- 15 Embed & polymerise overnight in oven at 60 °C , section with diamond knife as usual.