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TS Procure 812 - primary fixation Karnovsky's - transwells (TM - 013) V.2

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Protocol status: Working
We use this protocol and it's working

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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 All solutions are exchanged above and below the membrane, up to step 10.

3 Karnovsky's fixative: [M] 2.5 % volume 25% Glutaraldehyde and [M] 4 % volume 16% Paraformaldehyde **ProSciTech Catalog #**C004 in [M] 0.1 Molarity (M) Sorenson's Phosphate buffer (pH 7.2, 300mosmol/kg) for at least 00:40:00 or overnight 40m

4 [M] 0.1 Molarity (M) buffer 00:15:00 15m

5 [M] 1 % volume Osmium Tetroxide **ProSciTech** in buffer 01:00:00 1h

6 [M] 70 % volume Ethanol for at least 00:20:00 or overnight 20m

7 [M] 95 % volume Ethanol 00:20:00 20m

8 [M] 100 % volume Ethanol 00:20:00 20m

9 [M] 100 % volume Ethanol dried with molecular sieve 00:20:00 20m

Remove membrane

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

Conventional 5h 15m

11 [M] 50 % volume Ethanol [M] 50 % volume Propylene oxide **ProSciTech Catalog #C156** 00:20:00 20m

12 [M] 100 % volume Propylene oxide **ProSciTech Catalog #C156** 00:20:00 20m

13 [M] 50 % volume Propylene oxide **ProSciTech Catalog #C156** [M] 50 % volume Procure 812

14 [M] 100 % volume Procure 812 00:20:00 20m

15 [M] 100 % volume Procure 812 01:00:00 1h

16 Embed & polymerise overnight in oven at 60 °C , section with diamond knife as usual.