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Version 4

TS Spurrs - unstained FFPE on Thermanox coverslips (TM - 013) V.4

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Protocol status: Working

We use this protocol and it's working

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Abstract

Processing unstained FFPE sections on Thermanox coverslips to Spurr's resin block for EM.

Guidelines

All step times are the minimum time required. Longer steps are acceptable.

Protocol materials

⊗ xylene

⊗ xylene

⊗ xylene

⊗ Ethanol

⊗ Ethanol

⊗ Ethanol

⊗ Glutaraldehyde aqueous solution 25% EM Grade ampoules **ProSciTech Catalog #C002**

⊗ Osmium Tetroxide **ProSciTech**

⊗ Ethanol

⊗ ethanol

⊗ ethanol

⊗ ethanol

⊗ ethanol

Troubleshooting



HEADER

1 SAN:



SPEC No:

OPERATOR & STEPS:



OPERATOR & STEPS:

Dewax FFPE sections



15m

2  xylene or X3B  00:05:00

5m

3  xylene or X3B  00:05:00

5m

4  xylene or X3B  00:05:00

5m

Rehydrate

15m

5 [IM] 100 % volume  Ethanol  00:05:00

5m

6 [IM] 95 % volume  Ethanol  00:05:00

5m

7 [IM] 70 % volume  Ethanol  00:05:00

5m

Conventional - with reduced times for 5um thick FFPE section

2h 50m



- 8 [M] 2.5% % volume 20m
⊗ Glutaraldehyde aqueous solution 25% EM Grade ampoules **ProSciTech Catalog #C002**
in [M] 0.1 Molarity (M) Sorenson's phosphate buffer pH 07.2 300 mosmol/kg
⌚ 00:20:00 .
- 9 Wash buffer ⌚ 00:05:00 5m
- 10 [M] 1 Mass / % volume ⊗ Osmium Tetroxide **ProSciTech** in buffer ⌚ 00:30:00 30m
- 11 [M] 70 % volume ⊗ Ethanol ⌚ 00:10:00 10m
- 12 [M] 95 % volume ⊗ ethanol ⌚ 00:05:00 5m
- 13 [M] 100 % volume ⊗ ethanol ⌚ 00:05:00 5m
- 14 [M] 100 % volume dry ⊗ ethanol ⌚ 00:05:00 5m
- 15 [M] 100 % volume Spurrs: [M] 100 % volume dry ⊗ ethanol in 1:1 ratio 30m
⌚ 00:30:00
- 16 [M] 100 % volume Spurrs ⌚ 00:30:00 30m
- 17 [M] 100 % volume Spurrs ⌚ 00:30:00 30m
- 18 Oven polymerise 🔥 65 °C ⌚ Overnight