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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 Spurrs 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 [M] 100 % volume Ethanol 00:05:00 5m

6 [M] 95 % volume Ethanol 00:05:00 5m

7 [M] 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 30m

[M] 1 Mass / % volume Osmium Tetroxide **ProSciTech** in buffer 00:30:00

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 00:30:00 30m

16 [M] 100 % volume Spurrs 00:30:00 30m

17 [M] 100 % volume Spurrs 00:30:00 30m

18 Oven polymerise 65 °C Overnight