May 09, 2023

TS Spurrs - Cell Monolayer on Thermanox coverslips (TM - 013)

DOI
dx.doi.org/10.17504/protocols.io.n92ldpk17l5b/v1

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Protocol Citation: sandra.crameri 2023. TS Spurrs - Cell Monolayer on Thermanox coverslips (TM - 013). **protocols.io** https://dx.doi.org/10.17504/protocols.io.n92ldpk17l5b/v1

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

Created: May 09, 2023

Last Modified: May 09, 2023

Protocol Integer ID: 81635

Keywords: cell monolayer on thermanox coverslip, thermanox coverslip, cell monolayer, resin block for em, resin block, ts spurr, resin

Abstract

Processing a cell monolayer on Thermanox coverslips to Spurrs resin block for EM.

Guidelines

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

Protocol materials

- 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:

Conventional - with reduced times for monlayer thickness

2h 50m

2 [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 .

3 Wash buffer 00:05:00 — 5m

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

5 [M] 70 % volume Ethanol 00:10:00 — 10m

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

7 [M] 100 % volume ethanol 00:05:00 — 5m

8 [M] 100 % volume dry ethanol 00:05:00 5m

9 [M] 100 % volume Spurrs: [M] 100 % volume dry ethanol in 1:1 ratio 00:30:00 30m

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

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

12 Oven polymerise 65 °C Overnight