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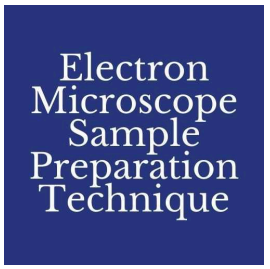
Electron microscope sample preparation technique_V2



Forked from Electron microscope sample preparation technique

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We use this protocol and it's working

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Abstract

There are eight main steps in preparing EM sample, including fixation, en bloc staining, agarose embedding, dehydration, infiltration, resin embedding, resin-embedded sample trimming and ultrathin sectioning.

This EM sample preparation protocol is established by Benjamin Padman and slightly modified by Wai Kit Lam and Runa Lindblom. Of note, this protocol is designed for processing cultured cell sample.

Attachments

PDF

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104KB

Materials

List of reagents

- 0.2 M Phosphate buffer (pH 7.4)
- 0.2 M sodium-cacodylate (NaCac) (pH 7.4)
- 16 % Paraformaldehyde solution, EM grade
- 25 % Glutaraldehyde solution, EM grade
- 2 % osmium tetroxide (OsO4)
- Potassium ferricyanide (K3Fe(III)(CN)6)
- Uranyl acetate (UA)
- Lead citrate
- Agarose, low-gelling point
- 70 %, 80 %, 90 %, 95 %, 100 % Ethanol
- Propylene oxide
- Dodecenylsuccinic anhydride (DDSA)
- Araldite 502
- Procure 812
- Benzyldimethylamine (BDMA)

Safety warnings

For hazard information and safety warnings, please refer to the SDS (Safety Data Sheet).

Fixation

1h

1 Aspirate cell culture media then fix cells with pre-warmed phosphate-buffered 4% Paraformaldehyde (PFA) at Room temperature for 01:00:00. 1h

2 Wash cells twice with [M] 0.1 Mass Percent sodium-cacodylate (NaCac) pH 7.4 buffer.

3 Post-fix the sample with [M] 2.5 Mass Percent glutaraldehyde in [M] 0.1 Mass Percent NaCac buffer for 24:00:00 at 4 °C. 1d

4 Wash cells three times with [M] 0.1 Mass Percent NaCac buffer.

5 Post-fix the sample with [M] 1 Mass Percent osmium tetroxide (OsO4), [M] 1.5 Mass Percent Potassium ferricyanide (K3Fe(III)(CN)6) in [M] 0.1 Mass Percent NaCac buffer On ice for 01:00:00. 1h

6 Bring the sample to BioWave Pro microwave (Pelco), and expose it to three microwave dutycycle (00:02:00 on, 00:02:00 off) at 100W under vacuum. 4m

7 Rinse cells three times with MilliQ water.

en bloc staining

8 Add [M] 2 Mass Percent uranyl acetate (UA) in MilliQ water to sample, followed by exposing the sample to three microwave duty-cycle (120s on, 120 s off) at 100W under vacuum.

Note

It is best to filter the 2% (w/v) UA using 0.22 µm pore size filter.

9 Rinse cells three times with MilliQ water.

Agarose embedding

10 Scrape and pellet (10000 x g, 00:03:00) cells in MilliQ water.

11 Resuspend the cell pellet in [M] 70 % volume Ethanol.

12 Centrifuge the sample again at 10000 x g, 00:03:00 .

13 Gently remove the supernatant (i.e. 70 % Ethanol).

14 Add one drop of low-melting point agarose to the cell pellet.

15 Vortex and centrifuge the sample immediately.

> Note
>
> The low-melting point agarose must be super-hot (close to 100°C) to achieve step 15.

16 Cut the solidified agarose-cell pellet into 1 mm cubes using a razor blade.

17 Transfer the agarose-cell pellet cubes to a flat-bottom crew cap tube.

Dehydration

40s

18 Perform a microwave assisted dehydration by graduated ethanol series (80 %, 90 %, 95 %, 100 %, 100 % (v/v); each at 150 W for 00:00:40). 40s

19 Perform a microwave assisted dehydration by propylene oxide (100%, 100% (v/v); each at 150 W for 00:00:40). 40s

Infiltration 3m

20 Infiltrate samples with Araldite 502/Procure 812 (Resin) by graduated concentration series in propylene oxide (25 %, 50 %, 75 %1 100 %, 100 % (v/v)); each at 250 W for 00:03:00 under vacuum). 3m

Note

The composition of the Resin is 51.7 % (w/w) DDSA, 18.6 % (w/w) Araldite 502, 26.3 % (w/w) Procure 812 and 3.4 % (w/w) BDMA.

Resin embedding

21 Put the infiltrated agarose-cell pellet cubes and the appropriate paper label into the resin-block mould, then fill up the mould with resin.

Note

It is important to remove all the bubbles. Usually, some bubbles are trapped under the paper label. It is best to use a pair of forceps to lift-up the paper label to release these bubbles, followed by using a 1 ml plastic transfer pipette to remove them.

22 Incubate the resin-embedded sample to a 60 °C oven for 48:00:00 or until the resin is fully polymerised. 2d

Resin-embedded sample trimming

23 Use a razor blade to carefully trim the resin block to expose the underlying agarose-cell pellet cubes.

24 Create a mirror surface on the agarose-cell pellet cubes using Ultra UCT ultramicrotome (Leica Biosystems) equipped with a glass knife.

Note

Before proceeding to the ultrathin sectioning, the block face must be cleaned, and the edges on the mirror surface must be parallel to each other.

Ultrathin sectioning

8m

25 Cut 70-90 nm sections on an ultramicrotome equipped with a diamond knife (Ultra 45° Diatome).

Note

Make sure you check the angle of the knife holder. For the Ultra 45° Diatome, the angle needs to be at 6°.

26 Load the ultrathin sections onto the grid. (Copper side up)

27 The grid is ready for TEM imaging.