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2D TEM CLEM (Correlative Light Microscopy and Electron Microscopy)

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Abstract

This protocol details the general procedure of Correlative Light Microscopy and Electron Microscopy (CLEM) with conventional chemical fixation and 2D Transmission Electron Microscopy (TEM) imaging.

Attachments

DOCX

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15KB

Materials

Materials

- 4% PFA
- 0.25% glutaraldehyde
- 2.5% glutaraldehyde
- imaging buffer
- 0.1 M sodium cacodylate
- 2% OsO_4
- 1.5% $K_4Fe(CN)_6$(Sigma-Aldrich)
- MiliQ water
- 2% aqueous uranyl acetate
- 50% etoh
- 75% etoh
- 95% etoh
- 100% etoh
- 50% Epon
- pure Epon 812
- microtome

poly-d-lysine-coated 35 mm Dish | No. 1.5 Gridded Coverslip (D) | 14 mm Glass Diameter **MatTek Corporation Catalog #**P35G-1.5-14-CGRD-D

Troubleshooting

General preparation:

6h 5m

1 Culture cells on poly-d-lysine-coated 35 mm MatTek dish (P35G-1.5-14-CGRD) and transfect the cells with the plasmids of the interest.

2 Pre-fix the cells in 37 °C -warmed 4% PFA and 0.25% glutaraldehyde in the imaging buffer.

3 Wash in the same imaging buffer.

3.1 Wash 00:05:00 in the same imaging buffer. (1/3) 5m

3.2 Wash 00:05:00 in the same imaging buffer. (2/3) 5m

3.3 Wash 00:05:00 in the same imaging buffer. (3/3) 5m

4 Image with a fluorescence microscopy, find the regions of the interest and their coordinates on the dish, then take the Z-stack fluorescence images and the grids map/coordinate by using phase contrast.

5 Fix the cells in 2.5% glutaraldehyde and [M] 0.1 Molarity (M) sodium cacodylate buffer for 01:00:00 . 1h

6 Wash in [M] 0.1 Molarity (M) sodium cacodylate buffer.

6.1 Wash in [M] 0.1 Molarity (M) sodium cacodylate buffer for 00:05:00 . (1/4) 5m

6.2 Wash in [M] 0.1 Molarity (M) sodium cacodylate buffer for 00:05:00 . (2/4) 5m

6.3 Wash in [M] 0.1 Molarity (M) sodium cacodylate buffer for 00:05:00 . (3/4) 5m

6.4 Wash in [M] 0.1 Molarity (M) sodium cacodylate buffer for 00:05:00 . (4/4) 5m

7 Fix in 2% OsO_4 and 1.5% $K_4Fe(CN)_6$ (Sigma-Aldrich) in [M] 0.1 Molarity (M) sodium cacodylate buffer for 01:00:00 . 1h

8 Wash in MiliQ water.

8.1 Wash in MiliQ water for 00:05:00 . (1/4) 5m

8.2 Wash in MiliQ water for 00:05:00 . (2/4) 5m

8.3 Wash in MiliQ water for 00:05:00 . (3/4) 5m

8.4 Wash in MiliQ water for 00:05:00 . (4/4) 5m

9 En bloc stained with 2% aqueous uranyl acetate Overnight at 4 °C . 5m

10 Wash in MiliQ water.

10.1 Wash in MiliQ water for 00:05:00 . (1/4) 5m

10.2 Wash in MiliQ water for 00:05:00 . (2/4) 5m

10.3 Wash in MiliQ water for 00:05:00 . (3/4) 5m

10.4 Wash in MiliQ water for 00:05:00 . (4/4) 5m

11 Dehydration in 50% etoh for 00:05:00 . 5m

12 Dehydration in 75% etoh for 00:05:00 . 5m

13 Dehydration in 95% etoh for 00:05:00 . 5m

14 Dehydration in 100% etoh.

14.1 Dehydration in 100% etoh for 00:10:00 . (1/3) 10m

14.2 Dehydration in 100% etoh for 00:10:00 . (2/3) 10m

14.3 Dehydration in 100% etoh for 00:10:00 . (3/3) 10m

15 Infiltration in 50% Epon for 0.5-1 hour.

16 Infiltration in pure Epon 812.

16.1 Infiltration in pure Epon 812 for 01:00:00 . (1/2) 1h

16.2 Infiltration in pure Epon 812 for 01:00:00 . (2/2) 1h

17 Add two drops of pure Epon into the center of the glass bottom of the culture dish.

18 Polymerize at 60 °C for 1-2 days.

19 Cell of interest were relocated based on the pre-recorded coordinates under a stereomicroscope.

20 Trim and cut ultrathin sections (50-60 nm) with microtome.

21 Observe the ultrathin sections in a electron microscope.