

Transmission Electron Microscopy (TEM) Protocol: Observation Details within Cells

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[Abstract] Transmission electron microscopy is a technique for observing the fine details of organelles in cells or tissues. This protocol is to be used to examine the membrane structure in cells with or without virus infection. Modifications should be made if users want to get images from tissues.

Materials and Reagents

1. Trypsin: working solution is 0.5% trypsin-0.2% EDTA (Life Technologies, Gibco®, catalog number: 15400-054)
2. NaH₂PO₄ (Kanto, catalog number: 37239-01)
3. Na₂HPO₄ (Chemical, catalog number: 37240-01)
4. Alcohol
5. Poly/Bed 812 (Electron microscopy sciences, catalog number: 14900)
6. Araldite 502 (Electron microscopy sciences, catalog number: 10900)
7. Dodecenylsuccinic anhydride (DDSA) (TAAB, catalog number: D025)
8. DMP-30 (Electron microscopy sciences, catalog number: 13600)
9. Propylene oxide (Merk, catalog number: S4912527-745)

Note: Toxic, need to handle with care and collect after use.

10. Syringe
11. Dropper
12. Grid (200 mesh) (TAAB)
13. 0.1 M Phosphate buffer (PB) (see Recipes)
14. Paraformaldehyde (4% in PB) (Bionovas, catalog number: AP0130-0500) (see Recipes)
15. Osmium tetroxide (1% in PB) (Electron microscopy sciences, catalog number: 51007) (see Recipes)

Note: Toxic, need to handle with care and collect after use.

16. Epon (see Recipes)
17. Toluidine Blue (Sigma-Aldrich, catalog number: NO202-146-2) (see Recipes)
18. Uranyl acetate (Art, catalog number: 8473) (see Recipes)
19. Lead citrate (TAAB, catalog number: L003) (see Recipes)

Equipment

1. Eppendorf tubes
2. Centrifuge
3. Vacuum drying oven
4. Diamond knife (Diatome)
5. Ultracut (Leica)
6. TEM (HITACHI, model: H-7100)

Procedure

Note: Put all droppers and syringes into the 60 °C oven the day before this procedure to remove all the water inside all stuffs.

1. After treatment, collecting cells (1×10^6) with 0.5% trypsin-EDTA and washing cell with 0.5 ml 0.1 M phosphate buffer (PB) in an eppendorf tube by centrifuge (300 x g, 5 min).
2. Add 0.5 ml paraformaldehyde (4% in PB) to fix cells in room temperature (RT) for at least 30 min (or store cells at 4 °C for long term storage).
3. Wash cells 3 times with 0.5 ml 0.1 M PB by centrifuge (300 x g, 5 min).
4. Add 0.5 ml Osmium tetroxide (1% in PB) into the tube and incubate cells for 1 h at RT.
5. Wash cells 3 times with 0.5 ml 0.1 M PB by centrifuge (300 x g, 5 min).
6. Dehydrate cells with adding 0.5 ml 70% alcohol for 5 min twice → centrifuge (300 x g, 5 min) → remove alcohol.
7. Dehydrate cells with adding 0.5 ml 85% alcohol for 5 min twice → centrifuge (300 x g, 5 min) → remove alcohol.
8. Dehydrate cells with adding 0.5 ml 95% alcohol for 5 min for three times → centrifuge (300 x g, 5 min) → remove alcohol.
9. Dehydrate cells with adding 0.5 ml 100% alcohol for 10 min for five times → centrifuge (300 x g, 5 min) → remove alcohol.

Note: From step 10, all materials used should be from 60 °C oven.

10. Add 0.5 ml propylene oxide into tubes and incubate for 3 min twice at RT. → centrifuge (300 x g, 5 min) → remove propylene oxide.
11. Add 1 ml (propylene oxide: Epon = 1:1) into tubes and incubate for 1 h at RT. → centrifuge (300 x g, 5 min) → remove Propylene oxide: Epon.
12. Add 1 ml pure Epon into tubes and put the samples into the vacuum drying oven to vacuum for 1 h at RT → remove Epon.
13. Add 1 ml pure Epon into tubes and vacuum for 1 h at RT. → remove Epon.
14. Add 1 ml pure Epon into tubes and vacuum overnight at RT.

15. Put tubes into 60 °C oven for at least 48 h.
16. Trimming-remove the edge of the block.
17. Get thick section (1 µm) with Ultracut using diamond knife→put the thick section on a slide and stain with 0.5% toluidine blue for around 40 s to 1 min (until the edge is dried)→ wash 1 min with water → observe under microscopy to find the cells.
18. Once we found the cells on the thick section, change the thickness of Ultracut to get thin sections (65-70 nm).
19. Put the thin sections on the grids (3-4 sections on one grid).
20. Stain sections with uranyl acetate and lead citrate for 3 min sequentially.
21. Wash the grids with water for 1 min and let them dry completely.
22. Get images from digital camera on TEM with identical magnificence.

Recipes

1. 0.1 M phosphate buffer (PB)
 - 19 ml 0.2 M NaH₂PO₄
 - 81 ml 0.2 M Na₂HPO₄
2. 4% paraformaldehyde
 - 4% paraformaldehyde in PB
3. Osmium tetroxide
 - 1% osmium tetroxide in PB
4. Epon
 - Use syringe to mix all reagents below to make Epon.
 - Poly/Bed 812 10 ml
 - Araldite 502 6 ml
 - DDSA 18 ml
 - DMP-30 0.7 ml
 - Epon could be stored in 4 °C or -20 °C for 1-2 weeks.
5. Toluidine Blue
 - 0.5% Toluidine Blue
 - 1% Sodium borate/in H₂O
 - Filter with 0.22 µm filter
6. Uranyl acetate
 - Saturated uranyl acetate in 50% Alcohol
7. Lead citrate
 - 1% lead citrate in H₂O
 - Add 3 drops of 10 M NaOH (around 0.5 ml)

Acknowledgments

This protocol is adapted from Chau and Lu (1996) and Lee *et al.* (2012).

References

1. Chau, Y. P. and Lu, K. S. (1996). [Differential permeability of blood microvasculatures in various sympathetic ganglia of rodents](#). *Anat Embryol (Berl)* 194(3): 259-269.
2. Lee, C. P., Liu, P. T., Kung, H. N., Su, M. T., Chua, H. H., Chang, Y. H., Chang, C. W., Tsai, C. H., Liu, F. T. and Chen, M. R. (2012). [The ESCRT machinery is recruited by the viral BFRF1 protein to the nucleus-associated membrane for the maturation of Epstein-Barr Virus](#). *PLoS Pathog* 8(9): e1002904.