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Transmission electron microscopy of fibroblasts

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

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Abstract

This protocol describes the procedure for preparing and imaging cultured cells by transmission electron microscopy used in the Seifert Lab.

Protocol materials

- 4% Paraformaldehyde solution
- Glutaraldehyde **Electron Microscopy Sciences Catalog #**16220
- Sorensens Phosphate Buffer **Electron Microscopy Sciences Catalog #**11600
- Osmium Tetroxide **Electron Microscopy Sciences Catalog #**19134
- 100% Ethanol
- Uranyl acetate **Electron Microscopy Sciences Catalog #**22400
- Lead Citrate (Reynolds) **Delta Microscopies Catalog #**11300

1 Fix cells by replacing the growth medium with a fixative solution containing 4% Paraformaldehyde solution and 3.5% EM-grade Glutaraldehyde **Electron Microscopy Sciences Catalog #**16220 in 0.1 M Sorensens Phosphate Buffer **Electron Microscopy Sciences Catalog #**11600 at 4 °C

2 Incubate for 00:05:00 at Room temperature

3 Replace solution with fresh fixative solution

4 Incubate for 01:00:00 at 4 °C

5 Wash cells thoroughly with 0.1 M Sorensens Phosphate Buffer **Electron Microscopy Sciences Catalog #**11600

6 Post-fix in 1% Osmium Tetroxide **Electron Microscopy Sciences Catalog #**19134 for 01:00:00 at 4 °C

7 Dehydrate sample in a graded ethanol series from 50% through 100% for 00:05:00 each

8 Wash twice with 100% Ethanol at 4 °C

9 Infiltrate with epoxy resin directly in the culture dish

10 Transfer into beam capsules and embed overnight

11 Section polymerized blocks at 80-90 nm using an ultramicrotome

12 Mount sections on 300-mesh copper EM grids

13 Stain with Uranyl acetate **Electron Microscopy Sciences Catalog #**22400 and Lead Citrate (Reynolds) **Delta Microscopies Catalog #**11300 to enhance contrast

14 Image on a transmission electron microscope