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Electron Microscopy with Primary Hippocampal Neurons

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We use this protocol and it's working

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Abstract

This protocol provides a reliable method for high-resolution electron microscopy of primary cultured hippocampal neurons, allowing detailed visualization of lipid droplets at synaptic terminals.

Guidelines

The protocol needs prior approval by the users' Institutional Animal Care and Use Committee (IACUC) or equivalent ethics committee.

Materials

Materials and Reagents:

- Poly-L-Ornithine (PLO) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**P3655
- Glutaraldehyde 50% EM Grade Aqueous **Electron Microscopy Sciences Catalog #**16320
- 2 mM $CaCl_2$: SigmaAldrich, 10043
- Sodium Cacodylate Buffer **Electron Microscopy Sciences Catalog #**11652
- 2% osmium tetroxide (OsO_4) (EMS)
- 1.5% potassium ferrocyanide [$K_4Fe(CN)_6$] (Sigma-Aldrich)
- 2% aqueous uranyl acetate (EMS)
- Embed 812 resin (EMS)
- Imidazole (Sigma Aldrich)
- PBS (phosphate-buffered saline)

Equipment:

- 5 mm cell culture cylinder
- Transmission electron microscope (Talos L 120C)
- 4k × 4k Ceta CMOS camera
- Velox software
- Ultramicrotome
- Humidified chamber

Neuronal Culture Preparation

1 Isolate and dissociate hippocampal neurons using standard dissection techniques.

2 Plate 38,000 neurons within 5 mm cylinders mounted on poly-ornithine coated glass coverslips.

3 Maintain neurons for 14-21 days under standard culture conditions (37 °C , 5% CO_2) until ready for fixation.

Fixation

1h 20m

4 Prepare a fixative solution containing 2.5% glutaraldehyde and [M] 2 millimolar (mM) $CaCl_2$ in [M] 0.1 Mass Percent sodium cacodylate buffer.

5 Fix the neurons by gently applying the fixative solution for 01:00:00 at Room temperature . 1h

6 Wash the fixed neurons 4 times with 0.1 M sodium cacodylate buffer containing 2 mM $CaCl_2$, 5 minutes per wash.

6.1 Wash the fixed neurons with [M] 0.1 Mass Percent sodium cacodylate buffer containing [M] 2 millimolar (mM) $CaCl_2$, 00:05:00 per wash. (1/4) 5m

6.2 Wash the fixed neurons with [M] 0.1 Mass Percent sodium cacodylate buffer containing [M] 2 millimolar (mM) $CaCl_2$, 00:05:00 per wash. (2/4) 5m

6.3 Wash the fixed neurons with [M] 0.1 Mass Percent sodium cacodylate buffer containing [M] 2 millimolar (mM) $CaCl_2$, 00:05:00 per wash. (3/4) 5m

6.4 Wash the fixed neurons with [M] 0.1 Mass Percent sodium cacodylate buffer containing [M] 2 millimolar (mM) $CaCl_2$, 00:05:00 per wash. (4/4) 5m

Post-Fixation 55m

7 Pre-incubate the cells in [M] 0.1 Mass Percent imidazole buffer for 00:05:00 . 5m

8 Fix the cells in 2% Osmium in [M] 0.1 Mass Percent imidazole buffer for 00:30:00 at Room temperature . 30m

9 Wash the cells in water 5 minutes x 4.

9.1 Wash the cells in water 00:05:00 . (1/4) 5m

9.2 Wash the cells in water 00:05:00 . (2/4) 5m

9.3 Wash the cells in water 00:05:00 . (3/4) 5m

9.4 Wash the cells in water 00:05:00 . (4/4) 5m

Staining 1h 20m

10 En bloc stain the neurons using 2% aqueous uranyl acetate for 01:00:00 at Room temperature or 4 °C Overnight . 1h

11 Allow the samples to stain thoroughly to enhance contrast for electron microscopy.

12 Wash the cells in water 5 minutes x4.

12.1 Wash the cells in water 00:05:00 . (1/4) 5m

12.2 Wash the cells in water 00:05:00 . (2/4) 5m

12.3 Wash the cells in water 00:05:00 . (3/4) 5m

12.4 Wash the cells in water 00:05:00 . (4/4) 5m

Dehydration and Embedding 1h 5m

13 Dehydrate the neurons through a graded ethanol series to prepare them for resin embedding (50%, 75%, 95% EtoH for 00:05:00 , 100% EtoH for 10 minutes x3). 5m

13.1 Dehydrate in 100% EtoH for 10 minutes. (1/3)

13.2 Dehydrate in 100% EtoH for 10 minutes. (2/3)

13.3 Dehydrate in 100% EtoH for 10 minutes. (3/3)

14 Infiltrate the cells in 50% of Embed 812 in EtoH for 01:00:00 then pure Embed 812 1 hour x2. 1h

15 Embed the neurons in Embed 812 resin according to the manufacturer's instructions.

Sectioning

16 Use an ultramicrotome (Leica) to cut ultrathin sections (50-60 nm thick) from the embedded samples.

17 Mount sections onto grids suitable for transmission electron microscopy.

Imaging

18 Place the sections in a Talos L 120C TEM microscope.

19 Operate the microscope at 80 kV to capture high-resolution images.

20 Use Velox software and a 4k × 4k Ceta CMOS camera for imaging.

Note

- All reagents for electron microscopy were acquired from EMS (Electron Microscopy Sciences), Hatfield, PA.
- Handle all chemicals, especially OsO_4 and uranyl acetate, with care and in a fume hood to prevent exposure to toxic fumes.
- Ultrathin sectioning requires practice; ensure sections are even and smooth for optimal imaging.