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Nerve tissue processing for transmission electron microscopy (TEM)

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ABSTRACT

Transmission electron microscopy (TEM) studies promote an improved understanding of functional studies and provide critical ultrastructural information. This document provide a step by step protocol to embed tissues for TEM analysis. First, the animals are transcardially perfused with 2% paraformaldehyde and 1.25% glutaraldehyde diluted in 0.12M Millonigs buffer (MB), pH 7.3, for 15-20 minutes. After dissection, the tissues are postfixed in the same fixative overnight at 4°C and then rinsed in MB 3 times for 30 minutes each. Embedding protocol includes osmication, dehydration, and embedding of tissues in plastic resin. The tissue blocks are trimmed and semithin sections (0.5 μm) are obtained and stained with 1% toluidine blue solution. Light microscopy image capturing is performed at 100X magnification for detailed analysis.

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KEYWORDS

TEM, embedding, plastic resin, morphometric analysis

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MATERIALS TEXT

MATERIALS

Propylene oxide **Electron Microscopy Sciences Catalog #20401**

Eponate 12 kit with DMP-30 **Ted Pella Inc. Catalog #18010**

37% Formaldehyde **Sigma Aldrich Catalog #252549**

Glutaraldehyde **Ted Pella Inc. Catalog #18431**

Ethyl Alcohol 200 Proof **Gold Shield Distributors**

Osmium Tetroxide **Electron Microscopy Sciences Catalog #19120**

Sodium phosphate monobasic **Vwr Catalog #0823-2.5KG**

Sodium phosphate dibasic **Contributed by users Catalog #0348-2.5KG**

Sodium chloride **Vwr Catalog #BDH9286-2.5KG**

Toluidine blue **Electron Microscopy Sciences Catalog #22050**

DPX Mounting Medium **Electron Microscopy Sciences Catalog #13510**

SAFETY WARNINGS

Osmium tetroxide (OsO_4) is VERY TOXIC. Work in the fume hood and use appropriate PPE.

nerve tissue collection

1 Animals are transcardially perfused with 0.12M Millonigs buffer (MB), ph=7.3, followed by 2% paraformaldehyde and 1.25% glutaraldehyde diluted in MB for **00:20:00**

2 After dissection, the tissues of interest are postfixed in the same fixative overnight at 4°C and then rinsed 3 times with PBS for **00:30:00 each**

3 Wash tissues 3 times with ddH2O for **00:10:00 each**

Fixation

4 Fix tissues with 1% Osmium solution diluted in ddH_2O for **01:00:00** (Place vials with tissue +Osmium solution in circular shaker)

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*Osmium is VERY TOXIC. Work in the fume hood and use appropriate PPE.

Prepare Osmium solution at least one day in advance.

5 Wash tissues 3 times with ddH_2O for **00:10:00 each**

Dehydration

6 Dehydrate tissues with alcohol solutions in different concentrations, as follow: 30%, 50%, 70, 80, 95, and 100% (twice) solution diluted in ddH_2O **00:10:00 each**

Use 200 Proof Alcohol to prepare the solutions.

7 Continuing the dehydration place 100% propylene oxide in the tissues for **00:20:00**

Infiltration

8 Infiltration step starts with tissues in 50% propylene oxide+ 50% Epon solution for 2 hours. After, place 100% Epon resin in vials overnight.

Epon Solution:
Use Eponate 12 Kit, with DMP-30 (Ted Pella #18010).
To prepare 100mL Epon: Mix 47.6mL of RESIN; 32.0mL of DDSA; 23.0mL of NMA; and 1.6mL of DMP-30

Embedding

9 Block tissue of interest in plastic molds. Place the tissue in a direction to obtain cross sections later.

Semi-thin sectionning

10 Cut semi-thin cross section (0.5µm) and place them on glass slides.

11 Label sections with 1% toluidine blue solution diluted in ddH2O for **00:00:30**

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12 Wash sections with ddH_2O, dry and coverslip with DPX medium.

Image acquisition

13 Acquire and analyze images (10X magnification for oveview image and 100X magnification for detailed image) using a Nikon Eclipse E600 microscope and Nikon camera DS-Fi3

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