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Chemical Fixation and embedding of cultured cells for Transmission Electron Microscopy

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DISCLAIMER

ABSTRACT

This protocol can be followed to fix primary cultured neurons (or cells in general) and embed them in epoxy resin. Later ultrathin sections can be obtained with an ultramicrotome for Transmission Electron Microscopy imaging. The reagents used in this protocol are toxic!

PROTOCOL CITATION

Marta Orlando 2021. Chemical Fixation and embedding of cultured cells for Transmission Electron Microscopy. **protocols.io** https://protocols.io/view/chemical-fixation-and-embedding-of-cultured-cells-bwsbpean

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GUIDELINES

work under chemical hood with safety protections

SAFETY WARNINGS

Please work under a chemical hood with golves and lab coat for all steps of this protocol!

glutaraldehyde, osmium tetroxide are highly toxic
also sodium cacodylate buffer contains arsenic and is toxic

uranyl acetate is radioactive

do not inhale/ingest/touch without gloves and read the safety information for each reagent before starting to work.

DISCLAIMER:

1 

Fix for 1 hr at RT in 1.25% glutaraldehyde in 66mM sodium cacodylate buffer

2 

Wash 3 times with 0.1 M sodium cacodylate buffer

3 

Postfix 1 hr in 1% OsO4 (optional: for better contrast add 1.5% K4Fe(CN)6 to the osmium solution) in 0.1M sodium cacodylate buffer

4 

Wash 3x10' with 0.1 M sodium cacodylate buffer

5 

Wash 3x10' with distilled H2O

6 

En bloc stain for 45' in 1% uranyl acetate solution in distilled H2O

7 

Wash 3x10' with distilled H2O

8 

Dehydrate by incubating in increasing EtOH concentrations: 10 minutes in 70%, 10 minutes in 80%, 10 minutes in 96%, 2x15 minutes in 100%

9 

Infiltrate in 30% Epoxy resin in EtOH

10 

10. Infiltrate in 70% Epoxy resin in EtOH

11 

11. Infiltrate in 90% Epoxy resin in EtOH

12 

12. Incubate in 100% Epoxy resin

13 

13. Flat embed in Epoxy resin

14 

14. Bake for 48 hrs at 65°C

15 15. Remove the glass coverslip from the resin block by thermal shock