

Dec 14, 2021

Intracardiac perfusion with fixative for ultrastructural neuroanatomical studies

Forked from a private protocol

DOI

<https://dx.doi.org/10.17504/protocols.io.b2ssqeee>

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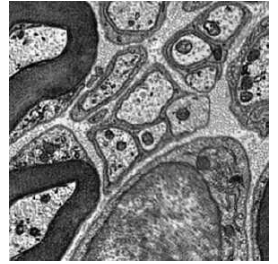
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Protocol Citation: Janet Keast, Peregrine Osborne 2021. Intracardiac perfusion with fixative for ultrastructural neuroanatomical studies. protocols.io <https://dx.doi.org/10.17504/protocols.io.b2ssqeee>

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Protocol status: Working

We use this protocol and it's working

Created: December 12, 2021

Last Modified: November 27, 2023

Protocol Integer ID: 55858

Keywords: tissue preservation, tissue fixation, electron microscopy, ultrastructure, intracardiac perfusion with fixative, intracardiac perfusion, ultrastructural neuroanatomical studies this protocol, tissues for ultrastructural neuroanatomical study, ultrastructural neuroanatomical study, anesthesia, peripheral nerve, brain in adult rat, preserving tissue, spinal cord, animal experimentation

Funders Acknowledgements:

NIH-SPARC

Grant ID: OT2OD023872

Abstract

This protocol is suitable for preserving tissues for ultrastructural neuroanatomical studies of peripheral nerves, ganglia, spinal cord or brain in adult rats. The protocol is performed under anesthesia and should incorporate all local requirements for standards of animal experimentation.

Materials

MATERIALS

 Ketamine **Lyppard Catalog #KETA11**

 Xylazine **Lyppard Catalog #L10605**

 Heparin sodium **Ellar Laboratories**

 Paraformaldehyde (prilled 95%) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #441244**

 Glutaraldehyde (25% aqueous solution EM grade) **ProSciTech Catalog #C001**

Preparation for perfusion

- 1 Make up the following solutions:

Perfusion prewash solution: To 300 ml 0.9% sodium chloride (w/v) add 3.75 ml 1% sodium nitrite (w/v) and 0.11 ml heparin (5000 IU/ml). This is made up immediately prior to use.

Perfusion fixative: 2% paraformaldehyde and 1.25% glutaraldehyde in 0.1M phosphate buffer, pH 7.4. The buffered paraformaldehyde solution is made up no longer than 48h prior to use, stored at 4C and brought to room temperature on the day of perfusion. Glutaraldehyde is added to this solution on the day of the perfusion.

Perfusion

- 2 Induce anesthesia by an intraperitoneal injection of ketamine (100 mg/kg) and xylazine (10 mg/kg).
- 3 After opening the chest cavity, inject the left ventricle with mixture of 0.25 ml heparin (5000 IU/ml) and 0.5 ml 1% sodium nitrite.
- 4 A needle connected to tubing (T-connector to prewash and perfusion solutions), and a peristaltic pump (setting: 50 ml/min) is then inserted into the left ventricle and clamped into place using hemostats. Make a small incision in the right atrium to drain blood and perfusate during the procedure.
- 5 Perfuse with pre-wash solution until the fluid flowing from the right atrium is clear, the liver and extremities are pale (typically 2-3 min).
- 6 Perfuse with fixative for 15-20 minutes, by which time the organs have stiffened and the neural tissues will be well preserved.
- 7 Dissect tissues required for analysis and place in fixative for a further 18-24h at 4C.

Post-perfusion

- 8 Wash tissues with phosphate-buffered saline (PBS; 0.1 M, pH7.2), 3 × 30 min washes.



- 9 Store in PBS at 4C until processed further for embedding and electron microscopy.