Dec 19, 2024

Intracardiac perfusion with buffer for anatomical studies

Forked from Intracardiac perfusion with fixative for anatomical studies

DOI
dx.doi.org/10.17504/protocols.io.3byl4kpp8vo5/v1

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Protocol Citation: Janet R Keast, Peregrine B Osborne, Nicole Wiedmann 2024. Intracardiac perfusion with buffer for anatomical studies. **protocols.io https://dx.doi.org/10.17504/protocols.io.3byl4kpp8vo5/v1**

Protocol status: Working

Created: November 01, 2021

Last Modified: December 19, 2024

Protocol Integer ID: 54689

Keywords: tissue preservation, tissue fixation

Funders Acknowledgements:
NIH SPARC
Grant ID: 3OT2OD023872

Abstract

This protocol is suitable for preserving tissues for anatomical studies of organs and major pelvic ganglia 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 #**KETAI1
- Xylazine **Lyppard Catalog #**L10605
- Heparin sodium **Ellar Laboratories**
- Paraformaldehyde fixative: 4% paraformaldehyde in phosphate buffered saline (PBS) **Contributed by users**

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.

Fixative: 4% paraformaldehyde in 0.1M phosphate buffer, pH 7.4. This is made up no longer than 48h prior to use, stored at 4C and brought to room temperature on the day of 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 Dissect tissues required for analysis and place in fixative overnight at 4 degrees.

Post-perfusion

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

8 Store in PBS containing 0.1% sodium azide at 4C until used for immunohistochemistry or other microscopic analysis.