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Intracardiac perfusion with fixative for anatomical studies

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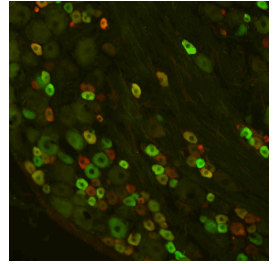
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Protocol status: Working

We use this protocol and it's working

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Protocol Integer ID: 31001

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Abstract


This protocol is suitable for preserving tissues for anatomical studies of organs, 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 #KETAI1**

 Xylazine **Lyppard Catalog #L10605**

 Heparin sodium **Ellar Laboratories**

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: 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 Perfuse with fixative for a minimum of 10 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 1h 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 containing 0.1% sodium azide at 4C until used for immunohistochemistry or other microscopic analysis.

