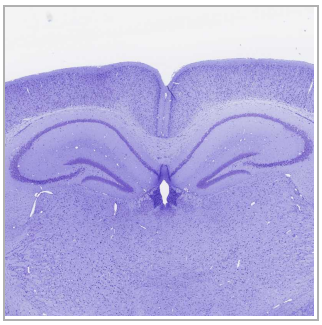

NOV 23, 2022

WORKS FOR ME 1

Intracardiac perfusion and brain fixation for immunohistochemistry

COMMENTS 0

DOI
dx.doi.org/10.17504/protocols.io.yxmvmk94og3p/v1

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ABSTRACT

This protocol aims to preserve brain tissue for immunohistochemistry studies. It is not valid for protein or RNA extraction studies.

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GUIDELINES

Read the whole protocol before starting the procedure. The whole process last about 3 days.

MATERIALS TEXT

20 mL Phosphate-Buffered Saline (**PBS**) or s**aline solution**

20 mL 4% Paraformaldehyde (**PFA**) prepared in **PBS** or Tris-Buffered Saline (**TBS**) (pH 7.2 – pH 7.4).

15 mL Falcon Tubes

30 mL Syringes

Winged infusion set

[M] 15 Mass / % volume Sucrose solution prepared in **PBS** or **TBS**.

[M] 30 Mass / % volume Sucrose solution prepared in **PBS** or **TBS**.

Dry ice

Metal Forceps

5 mL Falcon tubes or similar containers to store the brains

SAFETY WARNINGS

Fresh preparation of PFA is recommended. The solution can be stored at 4° for one month, or in aliquots at -80 for 3-4 months. PFA is toxic, handle it with care using gloves and goggles.

BEFORE STARTING

Prepare o filtered fresh PFA and saline solution (or PBS).

Animal Sacrifice

1 Before starting, fill a syringe with 20 mL of **ice-cooled PBS** and a separate syringe with 20 mL 4% Paraformaldehyde (**PFA**). Connect the **PBS syringe** to a Winged infusion set.

2 Anesthetize the animal deeply. Ensure there are no reflexes and breathing is slow. Carefully cut the thoracic cavity until the heart is exposed.

3 To perform intracardial perfusion, puncture the ventral region of the right ventricle with the winged infusion set and sustain the needle firmly. Carefully cut (make a small opening) the left atrium to facilitate fluid outflow. Perfuse the animal with 20 mL of cold On ice **PBS.** Change the syringe in the winged infusion set, and continue the perfusion with 20 mL of cold On ice **4% PFA.**

Note

1. Perfusion should be done at a **moderate-constant speed** to avoid rupturing the vascular system.

2. **Saline solution** instead of PBS is also suitable for this procedure as the objective is to clean the vascular system.

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3. If pulmonary swelling and outflow of solution through the nasal cavity are observed, the fluids might not travel through the vascular system properly. Traces of blood may still be present in the brain, and the intravascular fixation may not have been optimal.

4

When perfusion is finished, harvest the brain from the cranium, carefully removing the meninges to avoid damage to the tissue.

16h 8m 8s

Brain post-fixation and cryoprotection

16h

5 Submerge the brain in a 15 mL falcon tube (or similar) containing 12 mL of **4% PFA** for 16:00:00 at 4 °C .

Note

It is recommended that PFA volume is at least 10 times higher than the brain volume to achieve a penetration of **1mm/hour**. 15 mL Falcons allow most fluid to be above the brain and exert effective pressure. Avoid using containers where the brain is not submerged considerably in the fluid.

5m

6 Wash the brain 3 x 00:05:00 in the Falcon tube, with agitation, employing 4 °C **PBS** or **TBS** to remove PFA traces completely.

7 Thereafter, place 12 mL of [M] 15 Mass / % volume sucrose in the container and place it at 4 °C until the tissue sinks. At this point, the experimenter should see that the brain floats on the liquid surface.

Note

Brain sinking from [M] 15 Mass / % volume sucrose generally takes 6-8 hours

8 When the brain has sunk, discard the sucrose and add 12 mL of [M] 30 Mass / % volume sucrose. Place the tube again at 4 °C until the tissue sinks.

Note

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At his point, the brain generally sinks after 16-24 hours

Brain freezing

6m 8s

9 Prepare and label plastic or crystal containers to store the brain for the long-term at -80 °C. The containers and forceps to hold the brains must be cooled in **dry ice.**

2m

10 Discard the sucrose and extract the brain from the Falcon tube. Roll the brain on clean absorbent tissue paper to clean sucrose traces. Let the brain air-dry for 00:02:00.

4m 8s

11 To freeze the brain, place it on top of **aluminum foil** in a container filled with **dry ice** for 00:04:00. Alternatively, the brain can be wrapped in aluminum foil and submerged in liquid nitrogen for 00:00:08

Note

Prevent the brain from coming into contact with dry ice or liquid nitrogen to ensure proper tissue preservation.

12 Using the dry-ice-cooled forceps, place the brain into the dry-ice-cooled container and store it at -80 °C for further processing.

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