



DEC 14, 2023

OPEN ACCESS



DOI:
dx.doi.org/10.17504/protocols.io.6qpvr3rjbvmk/v1

Protocol Citation: Asta Zane, Nicole J Corbin-Stein, Gabrielle Childers, Jhodi Webster, Vickie Yang, Woong-Jai Won, Rajesh Gupta, Ashley Harms 2023. Mouse Perfusions. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.6qpvr3rjbvmk/v1>

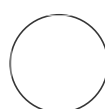
🌐 Mouse Perfusions

Gabrielle

Asta Zane¹, Nicole J Corbin-Stein¹, Childers¹,
 Jhodi Webster¹, Vickie Yang¹, Woong-Jai Won¹, Rajesh Gupta¹,
 Ashley
 Harms¹

¹University of Alabama at Birmingham, Department of Neurology, Center for Neurodegeneration and Experimental Therapeutics

ASAP Collaborative Research Network



Jhodi Webster

ABSTRACT

This protocol outlines the procedure to ethically euthanize/sacrifice a mouse using sterile, cold PBS and paraformaldehyde (4%). Following this perfusion, organs including the brain can be retrieved and fixed for further experiments.

MATERIALS

The fume hood

Protective gear (gloves, goggles, masks, lab coat)

Collecting dish

Styrofoam Surgical stage

Cold phosphate buffered saline (Millipore, catalog number: 6506-1L, diluted to 1x)

Cold Paraformaldehyde (PFA) solution 4% in PBS (Thermo scientific, catalog number: J19943-K2)

Heparin (Sigma, catalog number: H3149)

Two Syringes, 20ml (one for PBS, and one for PFA)

Two glass beakers

30 gauge infusion needle

Tissue dissecting scissors

Forceps

Micro spatula

Isofluorane box

MANUSCRIPT CITATION:

Williams GP, Schonhoff AM, Jurkuvenaite A, Gallups NJ, Standaert DG, Harms AS. CD4 T cells mediate brain inflammation and neurodegeneration in a mouse model of Parkinson's disease. *Brain*. 2021 Aug 17;144(7):2047-2059. doi: 10.1093/brain/awab103. PMID: 33704423; PMCID: PMC8370411.

Schonhoff, A.M., Figge, D.A., Williams, G.P. *et al.* Border-associated macrophages mediate the neuroinflammatory response in an alpha-synuclein model of Parkinson disease. *Nat Commun* **14**, 3754 (2023). <https://doi.org/10.1038/s41467-023-39060-w>

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working
We use this protocol and it's working

Created: Nov 03, 2023

Last Modified: Dec 14, 2023

PROTOCOL integer ID:
90382

Keywords: ASAPCRN

Funders**Acknowledgement:**

Co-pathologies Drive
Neuroinflammation and
Progression in PD
Grant ID: ASAP-021030

SAFETY WARNINGS

4% paraformaldehyde is toxic and flammable.

ETHICS STATEMENT

Perfusions must be done in a fume hood.

PROCEDURE

- 1 Pick the mouse up by its tail, place it into the isofluorane box, wait ~5 min. Assess if the mouse has reached a surgical plane of anesthesia by loss of response to toe pinch. Do not wait too long, since the heart may stop, and you need the heart beating to get a good perfusion.
- 2 Fill one beaker with PBS, add heparin to it (10 units per 100mL of PBS) and mix. Fill a second beaker with PFA solution.
- 3 From this step on, please operate in the fume hood. Place the mouse on its back. Firmly pin its four paws to a Styrofoam surgical station so that nothing moves during the perfusion procedure.
- 4 Connect the transfusion needle to a 20 ml syringe filled with PBS. Flush the tubing and needle to expel any air.
- 5 Perform transcardiac perfusion with PBS. Grip the skin on the chest with forceps and make an incision using tissue scissors to expose the abdominal cavity.
- 6 Grip the top of the skin with ophthalmic forceps and make lateral incisions beneath the ribcage using tissue scissors to expose the diaphragm and liver.
- 7 Carefully make incisions in the diaphragm along the entire length of the rib cage.
- 8 Make two cuts through both sides of the rib cage up to the collarbone using tissue scissors. Reflect the sternum up over the head of the mouse to fully expose the heart and lung
- 9 Carefully tear off pericardial sac and any other tissues covering the heart using dissecting forceps to

provide a clear view of the heart and vessels.

- 10 Secure the heart with dissecting forceps at a steady position. Insert the needle from the tip of the left ventricle at an angle approximately parallel to the midline of the heart. Do not push too hard and mind the angle to make sure the needle does not advance into the left atrium or right ventricle. The heart should be beating and one may observe backflow of blood into the needle before perfusion starts.
- 11 Make a small incision on the right atrium using scissors. Dark venous blood should flow out immediately. Start the PBS perfusion at once at a constant speed by manfully pushing the syringe. As PBS flushes out the blood, the liver should gradually turn pale.
- 12 Stop perfusion when the fluid flowing out is clear of blood. For adult mice, it takes ~10-15 ml saline.
- 13 Switch from saline to 4% PFA. Make sure no bubble gets into the perfusion system during the switch.
- 14 Steadily perfuse 4% PFA at a constant speed. As PFA goes into circulation, one can observe signs including body twitching, tail flicking and head moving. The tail standing up is a good sign of a good perfusion.
- 15 Perfuse ~10-15 ml 4% PFA for adult mouse. The whole mouse body should be stiff. Do not forget to flush your line with PBS before going to the next animal.
- 16 Unpin the mouse from the surgical station.
- 17 Decapitate the mouse with tissue dissecting scissors.

- 18 Remove the skin by first making an incision along the midline from the neck to the nose and then reflect the two flaps of skin rostrally and laterally to expose the skull.
- 19 Hold the head tightly with one hand. Trim off bones at the caudal end with dissecting scissors. Clear off any residual muscle on the skull with forceps or scissors. If the 4% PFA was done properly, the brain will shrink a little bit and there will be some space between the brain and the bones forming the foramen magnum.
- 20 Slide one blade of the fine scissors into the foramen magnum (underneath the brain) with the sharp side facing the bone on one side. Make a lateral cut. Repeat the cut on the contralateral side. Carefully remove the skull covering the cerebellum with ophthalmic forceps.
- 21 At the caudal end of the mid-sagittal suture, insert the tip of the fine tweezers between the foramen magnum and the brain. Make sure to disconnect the jaw bones from the skull as well. Rapidly lift skull away from the brain. Carefully remove the skull until the whole brain is exposed. An additional lifts may be necessary to expose the olfactory bulb.
- 22 Using a micro spatula to sever the nerve bundle on the ventral surface of the brain and scoop the brain out.