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Perfusion and Histology

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Ines Rodrigues-Vaz[1,2,3]

[1]Zuckerman Mind Brain Behavior Institute, Departments of Neuroscience and Neurology, Columbia University;
[2]Champalimaud Neuroscience Programme, Champalimaud Centre for the Unknown;
[3]Aligning Science Across Parkinson's (ASAP) Collaborative Research Network

Ines Rodrigues-Vaz

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We use this protocol and it's working

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Abstract

This is the standard protocol for perfusion and histology of brain slices for Rodrigues-Vaz and Athalye et al, 2025.

Materials

- 4% PFA
- PBS
- Paper towels
- Ensure to have enough isoflurane (fill to max line in holder on bottom right of regulator) for the duration of the surgery.
- Razor- ensure batteries are charged for hair removal
- Peristaltic pump and tubing
- Surgical tools for perfusion and brain dissection
- Needles
- Vials
- Antibodies:
  - conjugate anti-GFP: GFP Tag polyclonal antibody, Alexa Fluor 488 conjugate, Molecular Probes #A-21311
  - Anti-RFP antibody: Rabbit, Rockland, 600-401-379
  - Anti-Rabbit: Alexa Fluor 647, Invitrogen, A11011
- 1:1000 DAPI
- Mowiol solution
- Coverslips and Slides
- Nail polish
- Scanning microscope

Safety warnings

-Wear appropriate PPE as required by your institution.

Ethics statement

This protocol was approved by Columbia University IACUC. Please do not perform any of these procedures unless there is prior approval from the institution's animal ethics committee.

Before start

-Prepare 4% PFA solution, PBS, 0.05% Thimerasol-PBS and 0.4% Triton-PBS
-Prepare vials with 4% PFA for tissue collection and labels
-Prepare fume hood for cardiac perfusion – setup pump, tubing, solutions and tools required
-Turn on oxygen (~1.5 L/min)

Cardiac perfusion

1 Weigh mouse

2 Anesthetize mouse in isoflurane chamber (set 5% for deep induction). Monitor until the mouse is under deep anesthesia.

3 Remove the mouse from chamber and shave thorax (can use isoflurane nose tube to maintain anesthesia).

4 Test toe pinch reflex on both sides of animal before it is pinned down.

5 Pin down all four limbs of the animal and open thorax – carefully access the heart.

6 Needle connected to the peristaltic pump is inserted in the left ventricle and incision is made into the right atrium of the heart.

7 Pump is started with ice cold PBS followed by 4% PFA.

8 Brain is collected and stored in the fridge overnight in 4% PFA.

9 Brain is then moved into 0.05% Thimerasol-PBS and stored in the fridge until histological steps.

Histological preparation

10 Brains are sliced coronally at 50 mm using a vibratome (Leica VT1000) and collected onto 12-well plate.

11 Slices are washed multiple times with PBS for 5 minutes.

12 Followed by immunohistochemistry step – details described below:

- For GCaMP-GFP expression, sections are incubated with Alexa Fluor 488 conjugated GFP antibody diluted at 1:1000 in 0.4% Triton-PBS overnight at room temperature

- For td-Tomato expression, sections are incubated with primary anti-RFP antibody diluted at 1:2000 0.4% Triton-PBS overnight at 4^{o}C followed by secondary antibody anti-Rabbit Alexa Fluor 647 diluted at 1:1000 0.4% Triton-PBS at room temperature for 2 hours.

13 All sections are then counterstained with DAPI 1:1000 in PBS for 15 minutes.

14 Sections are then washed multiple times in PBS and mounted in glass slides and covered with Mowiol solution

15 Slides are allowed to dry overnight and sealed with nail polish.

Imaging of histological slides

16 Slides are imaged using an automated slide scanning microscope (AZ100) equipped with a 4X 0.4-NA objective (Nikon)

17 Automated high-throughput imaging is performed on custom-built automated slide scanner using a slide scanning microscope equipped with a 4× 0.4NA Plan Apo objective (Nikon Instruments Inc) and P200 slide loader (Prior Scientific), controlled by NIS-Elements using custom acquisition scripts (Nikon Instruments Inc.).

18 For detection of DAPI, Alexa Fluor 488 and Alexa Fluor 647 we used the following excitation wavelengths, respectively: 405 nm (filter 435/26 nm), 488 nm (filter 525/30 nm) and 647 nm (705/72 nm).

19 Imaging processing and analysis is done using Brain J.

Protocol references

Botta et al, 2020

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