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Immunohistochemistry & Microscopy-Falasconi & Kanodia et al 2025

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

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Abstract

Immunohistochemistry and microscopy methods used in Falasconi & Kanodia et al 2025.

Guidelines

Anesthesia:
Animals are anesthetized with a ketamine–xylazine solution.

Materials

Antibodies:

	A	B	C	D
	Chicken anti-GFP	Invitrogen	RRID: AB_2534023	Cat# A10262
	Rabbit anti-RFP	Rockland	RRID: AB_2209751	Cat# 600-401-379
	chicken anti-TH	Neuromics	RRID: AB_2201403	Cat# CH23006
	goat anti-ChAT	Millipore	RRID: AB_2079751	Cat# AB144P
	Donkey anti-rabbit Cy3	Jackson Immuno Research	RRID: AB_2307443	Cat#711-165-152
	Donkey anti-goat Cy5	Invitrogen	RRID: AB_2535864	Cat# A-21447
	Donkey anti-chicken 488	Jackson Immuno Research	RRID: AB_2340375	Cat#703-545-155
	Donkey anti-chicken Cy5	Jackson Immuno Research	RRID: AB_2340379	Cat#703-605-155
	Donkey anti-goat 488	Invitrogen	RRID: AB_2534102	Cat# A-11055

Safety warnings

Wear appropriate PPE as required by your institution.

Ethics statement

Prior ethics approval should be obtained before performing these experiments. Approval was obtained from the Cantonal Veterinary Office Basel-Stadt and performed in compliance with the Swiss Veterinary Law guidelines.

Before start

Read through the entire protocol before starting.

Transcardial Perfusion and Tissue Fixation

1 Prepare PBS and 4% PFA in PBS for perfusion.

2 Set up the perfusion pump and fill the tubing with PBS.

3 Pin the mouse to a Styrofoam plate.

4 Make a midline incision to expose the thoracic cavity, cutting through the skin and rib cage.

5 Insert the needle into the left ventricular chamber of the heart.

6 Switch on the perfusion pump and open the right atrium using sharp forceps or small scissors immediately.

7 Perfuse the mouse with PBS to flush out the blood.

8 Switch the perfusion to 4% PFA and perfuse until limbs are stiff.

9 Dissect the brain and spinal cord then place it in 10 mL of 4% PFA for post-fixation overnight at 4°C.

10 Transfer the brain to a 30% sucrose solution in PBS at 4°C until the tissue sinks (24–48 hours).

11 Embed the brain in OCT compound and freeze at -80°C.

Sectioning

12 Mount brain onto a cyrostat mount using OCT to be able to section in a coronal orientation.

13 Cut 80 µm sections and place sections in 24 well plates with PBS

Staining (IHC)

14 Incubate floating sections for 1 hour in blocking solution (1% BSA, 0.2% Triton X-100, PBS) at room temperature.

15 Add primary antibodies to the blocking solution and incubate for 1-3 days at 4°C.

16 Floating sections are washed x3 with PBS for 10 minutes each wash.

17 Add fluorophore-coupled secondary antibodies in blocking solution and incubate for 1 day at 4°C.

18 Floating sections are washed x3 with PBS for 10 minutes each wash.

19 Mount sections using fluorescence-compatible mounting medium and carefully place coverslips.

Imaging

20 For low-resolution overview imaging: scan slides with an Axioscan light microscope (Zeiss).

21 For higher resolution imaging use an Axio Imager M2 microscope (Zeiss) with a Yokogawa CSU W1 Dual camera T2 spinning disk confocal scanning unit.