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Protocol for Free-Floating Immunofluorescence Staining of Mouse Brain Sections

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We use this protocol and it's working

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Abstract

This protocol outlines the free floating immunofluorescence staining protocol for the detection of astrocytes, functional astrocyte markers and alpha-synuclein deposits in brain samples derived from 9 month old transgenic A53T mice.

Guidelines

Carry out all procedures in minimal light conditions to minimise signal bleaching.

Materials

Materials

Antibodies:

- GFAP: GFAP Polyclonal Antibody,Thermo #PA110004, Chk, IgY, 1:1000
https://www.thermofisher.com/antibody/product/GFAP-Antibody-Polyclonal/PA1-10004
- S100B: S100B Polyclonal Antibody, R&D Systems #AF1820, Gt, IgG, 1:1000
https://www.rndsystems.com/products/human-s100b-antibody_af1820
- ALDH1L1: ALDH1L1 Polyclonal Antibody, Thermo #PA578622, Rbt, IgG, 1:1000
https://www.thermofisher.com/antibody/product/ALDH1L1-Antibody-Polyclonal/PA5-78622
- Alpha Synuclein: Alpha-synuclein Monoclonal Antibody, BD Transduction Laboratories #610787, IgG1, 1:1000
https://www.bdbiosciences.com/en-au/products/reagents/microscopy-imaging-reagents/immunofluorescence-reagents/purified-mouse-anti-synuclein.610787
- CYP27B1: CYP27B1 Polyclonal Antibody, Sigma #ABN182, Rbt, , 1:1000
https://www.sigmaaldrich.com/AU/en/product/mm/abn182?srsltid=AfmBOor6baLssl6ia-64VxcYH707bW7EFKn9fbXQfvWy-f9L8-SnGPK3#product-documentation
- VEGFA: VEGFA Polyclonal Antibody, GeneTex #GTX102643, Rbt, IgG, 1:1000
https://www.genetex.com/Product/Detail/VEGFA-antibody/GTX102643?srsltid=AfmBOooiJAmhvDWhMXduxiN2hlhBbz87KtGYm98EA9h15f6uQ25bsvBr

	A	B	C
	Marker	**Primary antibodies**	**Secondary antibodies**
	GFAP	chicken anti-GFAP (Thermo #PA110004 1:1000)	Goat anti-chicken 488 (Thermo #A11039; 1:250) or Goat anti-chicken 594 (Thermo #A11042; 1:250) or Goat anti-chicken 647 (Thermo #A21449; 1:250)
	S100b	goat anti-S100b (R&D Systems #AF1820;	Donkey anti-goat 647 (Thermo #A21447; 1:250) or

	A	B	C
		1:1000)	Donkey anti-goat 594 (Thermo #A11058; 1:250)
	ALDH1L1	rabbit anti-ALDH1L1 (Thermo #PA578622; 1:1000)	Goat anti-rabbit 647 (Thermo #A32733; 1:250) or Goat anti-rabbit 594 (Thermo #A11012; 1:250)
	a-synuclein	Mouse anti-alpha-synuclein (BD #610787; 1:1000)	Goat anti-mouse 488 (Thermo #A11001; 1:250)
	VEGFA	Rabbit anti-VEGFA (Genetex #GTX102643;1:1000)	Goat anti-rabbit 594 (Thermo #A11012; 1:250)
	CYP27B1	Rabbit anti-CYP27B1 (Merck #ABN182; 1:1000)	Goat anti-rabbit 594 (Thermo #A11012; 1:250)

Equipment:

- Orbital Shaker
- Petri Dish
- Paintbrushes

Consumables:

- Superfrost + microscope slides
- Microscope slide coverslips (no. 1.5 thickness, 22×50mm)
- 6-well cell culture plates with mesh inserts

- Scintillation vials
- 30G x 1/2" 0.3mm x 13mm Hypodermic Needles

Key Reagents:

- Blocking Buffer: 4% BSA (w/v), 1% casein (w/v), 1.5% glycine (w/v), 0.25% Triton X-100 (v/v) in 1X PBS
- Triton X-100 (For 1X TBST 0.25%)
- Hoechst counterstain dye (Thermo #62249, 1ug/mL)
- Prolong Diamond Mounting Medium (Invitrogen #P36970)

Safety warnings

For hazard information and safety warnings, refer to the relevant SDS (Safety Data Sheet).

Ethics statement

Procedures involving the use of animals must receive approval from relevant animal ethics committees and must comply with institutional and national regulations

Before start

Sections described in this protocol are 30um free floating mouse brain sections, fixed with 4% PFA and cryopreserved in 30% sucrose in PBS. Prior to this procedure, sections are stored in antifreeze at -20 °C

Outline

1 Mouse brain tissue sections are washed initially to remove cryoprotectant, then blocking and primary antibody incubation overnight are carried out. Further washing is then followed by secondary antibody incubation and counterstaining, then the sections are mounted to microscope slides and coverslipped.

Tissue Preparation

20m

2 20m

1. Sections stored in a -20 °C freezer are removed and allowed to equilibrate to room temperature for 00:15:00 . .

2. Transfer sections into a mesh insert within a 6-well cell culture plate to separate them from the storage solution.

3. Move the mesh insert to the adjacent well containing approx. 5 mL 1X TBST 0.25% . Wash 3x with this solution for 00:05:00 per wash at Room temperature , changing solutions each wash. Agitate gently using an orbital shaker.

Blocking

1h

3 1h

1. Transfer sections from the final wash well into a new well containing IF blocking buffer and incubate at Room temperature for 01:00:00 on the orbital shaker on low speed

Primary Antibody Incubation

4 1. Prepare appropriate antibody solutions in scintillation vials by diluting primary antibody in IF blocking buffer. Prepare approx. 2 mL per vial .

2. Transfer sections from cell culture plate to the vial with appropriate antibody solution.

3. Place vial on orbital shaker and agitate lightly Overnight at Room temperature . Cover vial such that it is sheilded from light.

Secondary Antibody Incubation

2h 10m

5 3h 10m

**This and subsequent steps should be carried out in a darkened room if possible to avoid exposure to light.

1. Transfer sections from vial into well insert within a 6 well culture plate, and separate them from antibody solution.

2. Move the mesh insert to the adjacent well containing approx. 5 mL 1X TBST 0.25% . Wash 3x with this solution for 00:05:00 per wash at Room temperature , changing solutions each wash. Agitate gently using an orbital shaker.

3. Prepare new scintillation vial with appropriate secondary antibody solution, diluted in IF blocking buffer. Prepare approx. 2 mL per vial . Ensure the vial and solution are obscured from light.

4. Transfer sections from well insert to appropriate vial and incubate at Room temperature for 03:00:00 Agitate gently with orbital shaker and ensure vial is shielded from light.

5. Following incubation, transfer sections from vial into well insert and carry out 3x 00:05:00 washes with TBST 0.25% at Room temperature

Counterstain

6 10m

1. Prepare approx. 2 mL of 1ug/mL solution of Hoechst diluted in IF blocking buffer in a scintillation vial.

2. Transfer sections from well insert to vial and incubate for exactly 00:05:00

3. Place sections back into well insert and carry out 3x 00:05:00 washes with TBST 0.25% at Room temperature

Mounting and Coverslipping

1d 0h 10m

7 1. Transfer sections from well insert into a petri dish containing 1X TBST 0.25%. 1d 0h 10m

2. Take a microscope slide and insert it into the dish at an approx. 45° angle, allowing most of the surface of the slide to be submerged.

3. Using a fine paintbrush, gently move one section at a time onto the slide surface and orient it correctly. Ensure the tissue is flat with no folds. Hold each section in place and tilt the slide gently to remove excess solution and minimise slippage.

4. Repeat this process until all the sections are mounted. Allow all to dry for approx. 00:10:00

5. After slides have dried, apply 2-3 drops of Prolong Diamond Mounting Medium. Place a coverslip at one end and gently lower the other end of the coverslip with a 30G x 1/2" 0.3mm x 13mm Hypodermic Needle (or similar), ensuring no air bubbles form underneath. Gently press down to remove excess mounting medium.

6. Place slides in a slide box with the top of slide/coverslip/sections facing up and allow to dry for minimum 24:00:00 , or until mounting medium has completely hardened.

Protocol references

Giselle Sagredo, YuHong Fu, Hongyun Li 2023. Free floating immunofluorescent staining protocol on mouse brain sections. protocols.io https://dx.doi.org/10.17504/protocols.io.j8nlkoo55v5r/v2Version created by courtney.wright Wright