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Immunofluorescence Staining in Mouse Brain Tissue Sections

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We use this protocol and it's working

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Abstract

This protocol details the immunofluorescence staining in mouse brain tissue sections.

Materials

Pyrex® Staining Dish **Ted Pella Inc. Catalog #**36754-60

8-Section Staining Nets **Ted Pella Inc. Catalog #**36154-64

Fisherbrand™ Superfrost™ Plus Microscope Slides **Fisher Scientific Catalog #**12-550-15

ProLong™ Diamond Antifade Mountant **Invitrogen - Thermo Fisher Catalog #**P36961

Primary Antibodies:

	A	B	C	D
	Target	Species	Conc	Manufacturer
	TH	Rabbit	1:2000	Novus Biological N300109
	TH	Chicken	1:750	Abcam ab76442
	GFP	Chicken	1:2000	Aves Labs- GFP-1010
	GFP	Rabbit	1:500	Thermofisher A-11122
	GFP	Mouse	1:1000	Roche 11814460001
	P62	Guinea pig	1:2000	Progen GP62-C
	LAMP2	Rat	1:1000	Abcam ab13524
	GAD67	Mouse	1:500	Millipore Sigma MAB5406
	parvalbumin	Rabbit	1:500	Abcam ab11427
	TFE3	Rabbit	1:500	Abcam ab93808

Secondary Antibodies (From Thermofisher):

	A	B	C	D
	Antibody	Fluorophore	Concentration	Cat Number
	goat-anti rabbit	488	1:500	A-11008
	goat-anti rabbit	546	1:500	A-11010
	goat-anti rabbit	647	1:500	A-21245
	goat-anti mouse	488	1:500	A-11029
	goat-anti mouse	546	1:500	A-11003
	goat-anti rat	647	1:500	A-21247
	goat-anti chicken	488	1:500	A-11039
	goat-anti chicken	647	1:500	A-21449

Day 1

30m

1 Staining of 35µm free-floating mouse brain sections is performed in glass staining dishes (Pyrex 36754-60) using 8-section staining nets (Ted Pella 36154-64).
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1.1 The sections can be transferred between wells using a paint brush.

1.2 Volume of solution:

- 20 mL - 30 mL for antibodies.
- 50 mL for washing.

2 Wash sections 3 times (5 min each wash) in Phosphate Buffer Saline (PBS) at Room temperature to remove cryo-protectant solution.

2.1 Wash sections for 00:05:00 in Phosphate Buffer Saline (PBS) at Room temperature to remove cryo-protectant solution (1/3). 5m

2.2 Wash sections for 00:05:00 in Phosphate Buffer Saline (PBS) at Room temperature to remove cryo-protectant solution (2/3). 5m

2.3 Wash sections for 00:05:00 in Phosphate Buffer Saline (PBS) at Room temperature to remove cryo-protectant solution (3/3). 5m

3 Wash sections 3 times (5 min each wash) in PBS + 0.1% triton X-100.

3.1 Wash sections for 00:05:00 in PBS + 0.1% triton X-100 (1/3). 5m

3.2 Wash sections for 00:05:00 in PBS + 0.1% triton X-100 (2/3). 5m

3.3 Wash sections for 00:05:00 in PBS + 0.1% triton X-100 (3/3). 5m

4 Block sections for 01:00:00 in PBS + 10% Normal Goat Serum (NGS) + 0.4% Bovine Serum Albumin (BSA) and 0.2% triton X-100 for 01:00:00 at Room temperature . 2h

5 Incubate sections with primary antibodies in blocking solution (PBS + 0.1% triton X-100, 0.4% BSA) Overnight at 4 °C . 1h

Day 2 2d 2h 5m

6 Wash sections 3 times (10 min each wash) in PBS + 0.1% triton X-100 at Room temperature .

6.1 Wash sections for 00:10:00 in PBS + 0.1% triton X-100 at Room temperature (1/3). 10m

6.2 Wash sections for 00:10:00 in PBS + 0.1% triton X-100 at Room temperature (2/3). 10m

6.3 Wash sections for 00:10:00 in PBS + 0.1% triton X-100 at Room temperature (3/3). 10m

7 Incubate with fluorescent secondary antibodies (1:500) diluted in PBS + 0.1% triton X-100 + 0.4% BSA for 2 hours at Room temperature or 24:00:00 at 4 °C . 1d

> Note
>
> Sections should be protected from light at this step and all proceeding steps.

8 Wash sections 3 times (10 min each wash) in PBS + 0.1% triton X-100 at Room temperature .

8.1 Wash sections for 00:10:00 in PBS + 0.1% triton X-100 at Room temperature (1/3). 10m

8.2 Wash sections for 00:10:00 in PBS + 0.1% triton X-100 at Room temperature (2/3). 10m

8.3 Wash sections for 00:10:00 in PBS + 0.1% triton X-100 at Room temperature (3/3). 10m

9 Incubate with DAPI in PBS (1:5000) for 00:30:00 at Room temperature . 30m

10 Wash sections 3 times (10 min each wash) in PBS at Room temperature .

10.1 Wash sections for 00:10:00 in PBS at Room temperature (1/3). 10m

10.2 Wash sections for 00:10:00 in PBS at Room temperature (2/3). 10m

10.3 Wash sections for 00:10:00 in PBS at Room temperature (3/3). 10m

11 Mount sections:

11.1 Use a petri dish filled with PBS to mount the sections on superfrost plus slides (Fisher Scientific 12-550-15).

11.2 This is easiest to do using a medium sized paint brush.

11.3 Let sections dry 00:02:00 - 00:05:00 . 7m

Note

Coverslip using ProLongTM Diamond Antifade Mountant (Thermofisher P36961).

12 Dry slides for 24:00:00 at Room temperature . 1d

1. Protect slides from light at this step.
2. Store slides at 4 °C .