Apr 24, 2025

Immunofluorescence protocol for floating mouse brain sections

DOI
dx.doi.org/10.17504/protocols.io.kxygx31ddg8j/v1

Mary Alice Allnutt[1]

[1]Yale University

ASAP Collaborative Res...

N Nick C Buitrago-Pocasangre
Yale University

DOI: dx.doi.org/10.17504/protocols.io.kxygx31ddg8j/v1

Protocol Citation: Mary Alice Allnutt 2025. Immunofluorescence protocol for floating mouse brain sections. **protocols.io https://dx.doi.org/10.17504/protocols.io.kxygx31ddg8j/v1**

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

Created: November 01, 2023

Last Modified: April 24, 2025

Protocol Integer ID: 90932

Keywords: ASAPCRN

Funders Acknowledgements:
ASAP
Grant ID: ASAP-000529

Abstract

This protocol details the immunofluorescence for floating sections.

Attachments

nykhbkx77 (2).docx

23KB

Materials

Some of our preferred antibodies:

	A	B	C	D
	Host	**Target**	**Catolog No.**	**Recommended Dilution (Brain tissue)**
	Rabbit	pSer129 alpha-synuclein	Abcam ab51253	0.59722
	Mouse	Alpha-synuclein	BD Biosciences 610786	0.38889
	Guinea pig	GFAP	Synaptic systems 173 004	0.31944
	Rabbit	Iba1	Wako (019-19741)	0.25
	Goat*	ChAT	Millipore AB144P	0.38889

*use donkey serum and donkey-hosted secondary antibodies for all antibodies in an experiment with this primary

- Recombinant Anti-Alpha-synuclein (phospho S129) antibody **Abcam Catalog #**ab51253
- BD Transduction Laboratories™ Purified Mouse Anti-α-Synuclein **BD Biosciences Catalog #**610786
- GFAP antibody **Synaptic Systems Catalog #**173 004
- IBA Ab **Wako Catalog #**019-19741
- Goat anti-ChAT antibody **Merck Millipore (EMD Millipore) Catalog #**AB144P

Sectioning and long-term tissue storage

30m

1 Section the brains at 30 µm thickness on Leica cryostat (CM1860).

2 Collect the sections in cryoprotectant solution in 12-well plates and store at -20 °C.

3 To make 1000 ml cryoprotectant (**from IHCworld.com**):

	A	B	C
	a	Sucrose	300 g
	b	Polyvinyl-pyrrolidone (PVP-40)	10 g
	c	0.1M PB	500 ml
	d	Ethylene glycol	300 ml

e. Add PVP-40 to 0.1M PB (very slowly- this will take a while to dissolve). Stir to dissolve. Slowly add the sucrose to dissolve, and then add the ethylene glycol and bring the final volume to 1000 ml with 0.1M PB. Store at -20°C.

4 Wash the sections that are stored in cryoprotectant 3× 10 min prior to beginning and immunostaining protocols.

4.1 Wash the sections that are stored in cryoprotectant for 00:10:00 prior to beginning and immunostaining protocols (1/3). 10m

4.2 Wash the sections that are stored in cryoprotectant for 00:10:00 prior to beginning and immunostaining protocols (2/3). 10m

4.3 Wash the sections that are stored in cryoprotectant for 00:10:00 prior to beginning and immunostaining protocols (3/3). 10m

Immunofluorescence protocol for free-floating sections

8h 6m

5 10m

Note

Wash buffer: TBS + 0.1% Triton x-100 (Tx)

Equilibration: TBS + 0.1% Tx, 00:10:00 .

6 *Penetration*: TBS + 0.5% Tx, 00:20:00 . 20m

7 Wash 3× 2 min.

7.1 Wash for 00:02:00 (1/3). 2m

7.2 Wash for 00:02:00 (2/3). 2m

7.3 Wash for 00:02:00 (3/3). 2m

8 *Neutralization*: [M] 0.3 Molarity (M) Glycine in TBS + 0.1% Tx, 00:30:00 . 30m

9 Wash 3× 2 min.

9.1 Wash for 00:02:00 (1/3). 2m

9.2 Wash for 00:02:00 (2/3). 2m

9.3 Wash for 00:02:00 (3/3). 2m

10 *Blocking*: 3% goat serum in TBS + 0.1% Tx, 01:20:00 . 1h 20m

11 Wash 3× 2 min.

11.1 Wash for 00:02:00 (1/3). 2m

11.2 Wash for 00:02:00 (2/3). 2m

11.3 Wash for 00:02:00 (3/3). 2m

12 *Primary incubation:*

12.1 Prepare in 1% goat serum in TBS + 0.1% Tx.

12.2 Overnight 4°C, shaking. 1h 20m

13 Wash 3-4× 2 min.

13.1 Wash for 00:02:00 (1/4). 2m

13.2 Wash for 00:02:00 (2/4). 2m

13.3 Wash for 00:02:00 (3/4). 2m

13.4 Wash for 00:02:00 (4/4). 2m

14 *Secondary incubation*:

14.1 Prepare in 1% goat serum in TBS + 0.1% Tx.

14.2 1:500 for 04:00:00 at Room temperature . 4h

15 Wash 3-4× 2 min with TBS + 0.1% Tx and then twice with TBS alone.

15.1 Wash for 00:02:00 with TBS + 0.1% Tx and then twice with TBS alone (1/4). 2m

15.2 Wash for 00:02:00 with TBS + 0.1% Tx and then twice with TBS alone (2/4). 2m

15.3 Wash for 00:02:00 with TBS + 0.1% Tx and then twice with TBS alone (3/4). 2m

15.4 Wash for 00:02:00 with TBS + 0.1% Tx and then twice with TBS alone (4/4). 2m

16 Quench autofluorescence (optional):

16.1 Dip sections briefly in ultrapure H_2O.

16.2 Incubate in $CuSO_4$ buffer ([M] 10 millimolar (mM) $CuSO_4$ in [M] 50 millimolar (mM) ammonium acetate buffer, pH 5) for 00:15:00 at Room temperature . 15m

16.3 Dip sections in ultrapure water again briefly prior to mounting.

> **Note**
>
> Sections may appear slightly wrinkled after this treatment, but should still be able to be mounted without issue.

17 Mounting

17.1 Fill petri dish with TBS. Have serological pipette and secondary container available.

17.2 Place a clean slide in petri dish diagonally, with label resting against the side of the dish, above the level of the solution, and the rest submerged.

17.3 Using fine detail paint brush, lift floating section and gently adhere to slide near the top of the TBS. Can gently tap the section to get it to stick to the slide, or lift one corner of the section along the slide slightly above the water level to help it stick.

17.4 Using serological pipette, gently remove a few mL of TBS, leaving the section adhered to the slide.

17.5 After all sections are mounted, allow excess TBS to dry and then mount using Vectashield mounting medium with DAPI and a glass coverslip.

Note

Can replace TBS with PBS if not using antibodies targeting phosphorylated proteins.