Free floating immunofluorescence protocol on mouse brain sections for tau pathology

Felicia Suteja[1], Hongyun Li[1], YuHong Fu[1]

[1]University of Sydney

Team Kirik

courtney.wright

FEB 18, 2024

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.6qpvr3j23vmk/v1

Protocol Citation: Felicia Suteja, Hongyun Li, YuHong Fu 2024. Free floating immunofluorescence protocol on mouse brain sections for tau pathology. **protocols.io** https://dx.doi.org/10.17504/protocols.io.6qpvr3j23vmk/v1

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

ABSTRACT

This protocol describes our free-floating multiplexed immunofluorescent staining protocol to ascertain levels of tau and phospho- tau in mouse tissue from transplanted human iPSC cells carrying different PD related mutations.

GUIDELINES

IMPORTANT: perform all antibody incubation steps and steps following in minimal light so as not to bleach signals prior to imaging

Created: Feb 18, 2024

Last Modified: Feb 18, 2024

PROTOCOL integer ID: 95382

Keywords: ASAPCRN

Funders Acknowledgement:
Michael J Fox Foundation
Grant ID: ASAP-000497

MATERIALS

Equipment

- Orbital shaker
- black porcelain spot plate

Consumables

- microscope slides
- 6-well plates and net inserts
- Microscope slide coverslips (no. 1.5 thickness, 22x50mm)

1st	AT8(Ms IgG1)	SP70 (rb)	TH (Ms IgG2b)
Cat#	Thermofisher #MN1020	Sigma #SAB5500182	Thermofisher # TA506549
dilution	200 (400 if 1:1 with glycerol)	200	200

Primary antibody details

A	B	C	D	E
2nd	Gt @ Ms IgG1	Dn@Rb-AF568	Gt@Ms IgG2b-AF647	Hoechst 33,342 (1mg/ml stock)
Cat#	ThermoFisher #A-21206	ThermoFisher #A-10042	Thermofisher #A-#A-21242	Sigma #B2261
dilution	250	250	200	1:1000

Secondary antibody details

Homemade IF blocking buffer (NDS)

- 2% Donkey serum (Sigma, D9663)

- 1% BSA (best with IgG-free and protease-free) (Sigma, A9085 or JIR #001-000-173).
- 0.2% TritonX-100 (Sigma, T9284).
- 0.1% gelatine (from fish skin, Sigma, G7041).
- 0.1% Tween-20 (Sigma, P1379).
- 0.01% Sodium Azide (Sigma, S2002)

in 1XPBS, aliquoted and store at -20°C.

SAFETY WARNINGS

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

Experimental outline

1 Briefly, the mouse brain tissue sections are prepared by washing off the cryoprotectant medium and then antigen retrieval is performed followed by quenching, blocking and primary antibody incubation. Sections are then washed and incubated in the appropriate secondary antibody solution and are then mounted, cover-slipped and sealed.

Day 1 - Tissue prep

5m

2 30 µm mouse brain sections were stored in anti-freeze solution at -20 °C until required. 5m

1. Pour sections into a well insert in a 6-well plate to separate storage solution from section
2. Move the well insert to another well containing approximately 6 mL of 1x PBS. Wash at least 6x with 1x PBS for 00:05:00 each on an orbital shaker using low speed at

Antigen retrieval

27m

3 27m

1. Place sections in labelled glass vials containing 6 mL 1x citric buffer (CB) pH6.0.
2. Place in the steamer (Breville, Model: BFS800BSS) on high for 00:22:00 98 °C.
3. Cool down to Room temperature
4. Place the sections back into its corresponding wells.
5. 1xPBS wash: 2 x 00:05:00

Blocking and primary incubation

3d 2h

4 3d 2h

1. Incubate in blocking buffer: 02:00:00 Room temperature on shaker 60 rpm
2. Make primary antibody cocktail in home-made IF buffer
3. Label ceramic plate and place sections in antibody cocktail: 72:00:00 at 4 °C on the shaker

- AT8(Ms IgG1), Thermofisher #MN1020 1:200
- SP70 (rb), Sigma #SAB5500182 1:200
- TH (Ms IgG2b), Thermofisher # TA506549 1:200

Day 4 - Secondary antibodies

2h 25m

5 2h 25m

1. Transfer sections from ceramic plate to well plates
2. 0.1% PBST wash: 3 x 00:10:00
3. Make secondary cocktails as below in IF blocking buffer

- Gt @ Ms IgG, ThermoFisher #A-21206 1:250
- Dn@Rb-AF568, ThermoFisher #A-10042 1:250
- Gt@Ms IgG2b-AF647, Thermofisher #A-#A-21242 1:200
- Hoechst 33,342 (1mg/ml stock) Sigma #B2261 1:1000

4. Place sections and antibody cocktails in a ceramic plate: 02:00:00 Room temperature on the shaker 60 rpm
5. Place sections back into wells
6. 0.1% PBST wash: 3 x 00:10:00
7. 1x PBS wash: 3 x 00:05:00

Mounting and sealing

5m

6 5m

1. Label slides with corresponding section and antibody information
2. Place sections in a petri dish with 1xPBS and nudge the sections onto slides with brush
3. Mount with anti-fade media (DAKO Fluorescence Mounting Medium, Agilent, cat# S302380-2)
4. Wait for sections to become dry and seal with sealant (Biotium CoverGripTM Coverslip Sealant, cat#23005)
5. Store in slide box and store in fridge/cool room 00:05:00