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Fluorescent Immunolabelling for Alpha-Synuclein in neuronal primary culture (Testing PFF toxicity)

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We use this protocol and it's working

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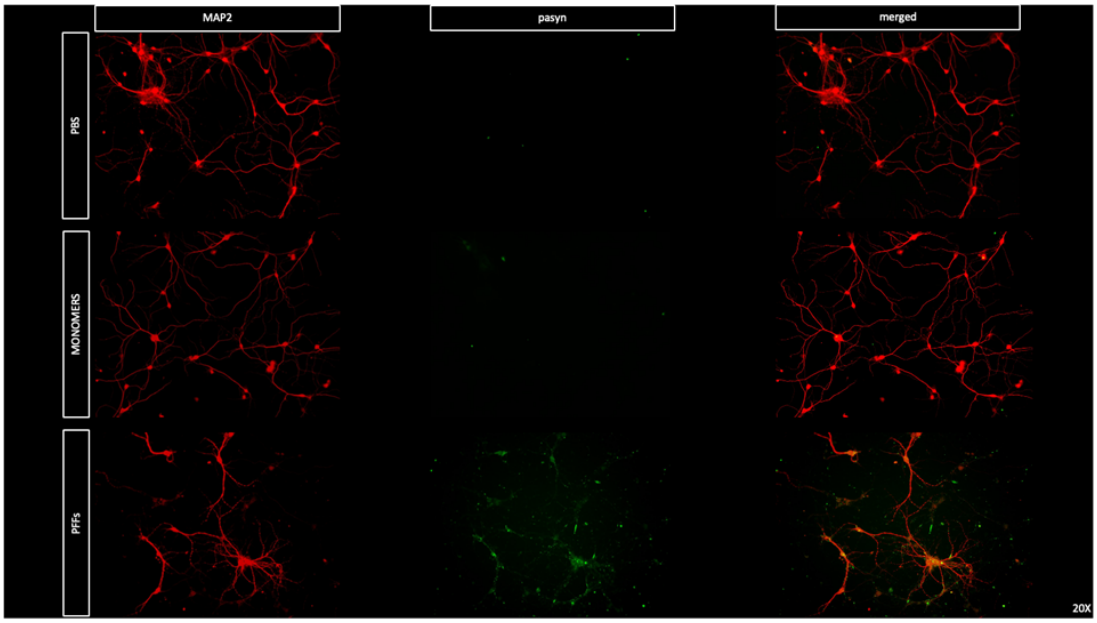
Abstract

This protocol is designed to perform fluorescent immunolabelling on neuronal primary culture after PFF incubation.
The labelling of phosphorylated Alpha-synuclein is considered as a marker of the PFF toxicity and should be performed to test the PFF before *in vivo* injection.

See protocol for PFF preparation
https://www.protocols.io/view/production-of-alpha-synuclein-preformed-fibrils-pf-b39rqr56

and protocol for neuronal primary culture
https://www.protocols.io/view/primary-culture-cortical-hippocampal-neurons-e15-1-cfiztkf6



Fluorescent immunolabelling of phosphorylated Alpha Synuclein (green) in neuronal primary culture.

Image Attribution

image by Sabina Marciano

Materials

Reagents:

- Purified anti-α-Synuclein Phospho (Ser129) Antibody **BioLegend Catalog #**MMS-5091
- Anti-MAP2 antibody (ab5392) **Abcam Catalog #**ab5392
- Donkey Anti-Mouse IgG H&L (Alexa Fluor® 488) **Abcam Catalog #**ab150105
- Donkey anti-Chicken IgY (H L) Highly Cross Adsorbed Secondary Antibody Alexa Fluor™ 594 **Invitrogen - Thermo Fisher Catalog #**A78951

Solutions:

4% (wt/vol) Paraformaldehyde/4% (wt/vol) sucrose/PBS
For 50 ml in PBS, pipette 10 ml of 20% paraformaldehyde into ~40 ml of PBS, add 2 g of sucrose and mix it until it dissolves. Bring the volume to 50 ml with PBS. This solution works best if it is used at room temperature, although others report optimal results when it is warmed to 37degrees. This solution can be stored at 4 degrees celsius, protected from light, for 1 week.

PBS/BSA0.005%/TWEEN0.05%
Dissolve 1mg BSA in 10mL PBS. Use 10uL of this solution for 20mL of PBS. Add 10uL Tween 20. Prepare this fresh on the day of use. All the washing steps are done for 10min.

1 Aspirate the medium from coverslips (aspirate from only a few coverslips at a time).

2 Add 4% (wt/vol) PFA /4% (wt/vol) sucrose (1 ml for a 12-well plate).

3 Incubate the plate at Room temperature for 00:15:00 . 15m

4 Wash 3 times 00:10:00 with PBS/BSA/TWEEN solution. 10m

5 Proceed or store in the fridge in PBS, protected from light, for up to 1 week.

6 Set up humidifier chambers.

7 Incubate with 0.5% Triton in PBS/BSA/TWEEN for 00:05:00 . 5m

8 Wash 3 times 00:10:00 with PBS/BSA/TWEEN. 10m

9 Block in Donkey Serum 1:20 in PBS for 00:30:00 . 30m

10 During the blocking, prepare the primary antibody solution.
In PBS/BSA/TWEEN, add Biolegend psyn (MMS-5091) Mouse (1:1000) and Abcam MAP2 (ab5392) chicken Millipore (1:2000).

11 Incubate with primary antibody for 01:00:00 at Room temperature . 1h

12 Wash 3 times 00:10:00 with PBS/BSA/TWEEN. 10m

13 During the washes, prepare the secondary antibody solution:

In PBS/BSA/TWEEN, add Alexa Fluor 488 donkey anti-mouse (1:1000) + Alexa Fluor 594 donkey anti-chicken (1:1000).

14 Incubate in secondary antibody solution for 01:00:00 at Room temperature in the DARK. 1h

15 Wash 3 times 00:10:00 with PBS/BSA/TWEEN. 10m

16 Incubate with DAPI 1:10,000 in PBS 00:10:00 at Room temperature. 10m

17 Wash 3 times 00:10:00 with PBS. 10m

18 Mount the coverslips onto glass slides with Prolong Gold mounting medium.