



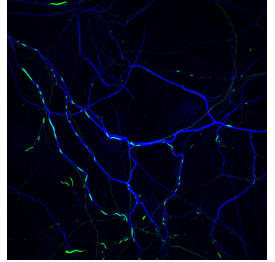
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Version 1

## 🌐 Fixation and immunocytochemistry (fluorescence) in PFF-treated cultures V.1

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**We use this protocol and it's working**

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## Abstract

This protocol outlines methods used for the preparation of in vitro models for fluorescent imaging including primary rat and mouse hippocampal cultures treated with PFFs.

## Image Attribution

Maria Iuliano -60x image of rat hippocampal neurons (DIV28) after 7-day treatment with PFFs. Map2 (Blue) and phopsho-a-syn Ser129 (green)

## Guidelines

Parameters used in this protocol may be optimized and modified for individual use depending on experimental needs.



## Materials

1% SDS for PFF inactivation

4% PFA 4% sucrose in 1x PBS

1x PBS

ICC Buffer: 5% NGS in 1x PBS

ICC Blocking Buffer: 5% NGS 0.3% Triton-X in 1x PBS\

Primary & secondary antibodies of choice

Microscope slides

Forceps

Mounting medium of choice

## Protocol materials

 Aqua-Poly/Mount **Polysciences, Inc. Catalog #18606-100**

## Safety warnings

- ⚠ When working with PFFs, it is best to follow proper safety precautions and wear appropriate PPE, including lab coat, gloves, sleeve guards, safety glasses or face shield, and N95 or FFP2 mask.

Inactivation and disposal of PFFs and PFF-contaminated items can be done using 1% SDS as described in literature.

Collect and dilute solutions into 10vol 1% SDS for  01:00:00 at  Room temperature

Discard used tips into primary collection container with 1% SDS

Inactivated liquid can be discarded with liquid waste

Discard inactivated items in primary container into biohazard

Wipe surfaces or contaminated tools down completely with 1% SDS, followed by water, then EtOH

### Citation

Bousset L, Brundin P, Böckmann A, Meier B, Melki R (2016)

. An Efficient Procedure for Removal and Inactivation of Alpha-Synuclein Assemblies from Laboratory Materials.

<https://doi.org/10.3233/JPD-150691>

LINK

## Before start

Please read safety guidelines ahead of working with PFFs



## Fixation and Immunocytochemistry (Fluorescence)

4h 30m

- 1 In a cell culture hood remove media and wash briefly with 1x PBS.

2m

### Safety information

Collect media and first wash as PFF waste for inactivation. Once in fixative, plate(s) can be moved to benchtop.

- 2  20 rpm, Room temperature , 00:15:00 Fix using 4% PFA 4% sucrose in 1xPBS with gentle rocking/shaking.

15m

### Note

Check whether the antibodies you intend to use require specific fixation. Optimization may be needed.

- 3  20-30 rpm, Room temperature , 00:10:00 Wash 1x PBS. Repeat 3 times.

30m

### Note

Pause Point: Coverslips can be stored after fixation for up to 2 weeks (or maybe more), though immediate processing is ideal. Store at 4C away from light, with the plate wrapped in parafilm to reduce evaporation.



- 4  20-30 rpm, Room temperature , 00:45:00 Block in blocking buffer (5% NGS, 0.3% Triton-X in 1x PBS)

45m

- 5  20 rpm, 4°C  Overnight Incubate in solution containing primary antibodies diluted in 5% NGS in 1x PBS



## Note

### Antibodies

|  | A                               | B                | C         |
|--|---------------------------------|------------------|-----------|
|  | Antibody                        | Company          | Catalog # |
|  | Gephyrin                        | Synaptic Systems | 147111    |
|  | HA (HA-tag)                     | BioLegend        | 901513    |
|  | HA (HA-tag)                     | Cell Signaling   | 3724      |
|  | Homer                           | Synaptic Systems | 160003    |
|  | MAP2                            | Millipore        | AB5543    |
|  | MAP2                            | Millipore        | MAB364    |
|  | Neurofilament                   | Millipore        | AB5539    |
|  | Phospho- $\alpha$ -syn (Ser129) | Biolegend        | 825701    |
|  | VGAT                            | Synaptic Systems | 131003    |
|  | VGLUT1                          | Millipore        | AB5905    |

Some commonly used antibodies in our lab for PFF-treated cultures. Dilutions may need to be optimized to individual needs.

6  20-30 rpm, Room temperature , 00:10:00 Wash 1x PBS. Repeat 3 times.

30m

7  20 rpm, 4°C, 02:00:00 Incubate in solution containing secondary antibodies diluted in 5% NGS in 1x PBS

2h

## Note

Secondary incubation can vary (up to O/N), but typically 2-3hr at 4C works well. 1hr at RT can also work, though it may result in excess background signal, depending on the antibody

Typically, we use Alexa Fluor secondaries from Life Technologies, except for anti-chicken CF 405 (Millipore SAB4600466).



- 8  20-30 rpm, Room temperature , 00:10:00 Wash 1x PBS. Repeat 3 times. 30m
- 9 Mount coverslips onto microscope slides using  Aqua-Poly/Mount **Polysciences, Inc. Catalog #18606-100** or preferred mounting medium. 5m
- 10 Let cure  48:00:00 before imaging 2d

## Citations

Bousset L, Brundin P, Böckmann A, Meier B, Melki R. An Efficient Procedure for Removal and Inactivation of Alpha-Synuclein Assemblies from Laboratory Materials.

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