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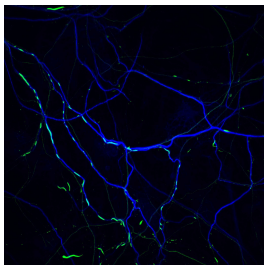
Fixation and immunocytochemistry (fluorescence) in PFF-treated cultures V.1



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ASAP Collaborative Rese… Maria's Workspace

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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 Step 9

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..
LINK
https://doi.org/10.3233/JPD-150691

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-α-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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