

JUN 15, 2023

## OPEN ACCESS

## DOI:

dx.doi.org/10.17504/protocol s.io.eq2lyjzomlx9/v1

**Protocol Citation:** Maryana Nissan, Divya.D.A Raj 2023. Immunofluorescent labeling of cultured cells . **protocols.io** 

https://dx.doi.org/10.17504/protocols.io.eq2lyjzomlx9/v1

License: This is an open access protocol distributed under the terms of the Creative Commons
Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

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

Created: Jun 15, 2023

Last Modified: Jun 15, 2023

**PROTOCOL** integer ID:

83495

**Keywords: ASAPCRN** 

1 Wash cells with 1X Phosphate-Buffered solution [1X PBS].

## Immunofluorescent labeling of cultured cells

Maryana

Nissan<sup>1</sup>, Divya.D.A Raj<sup>1</sup>

<sup>1</sup>Northwestern University

Maryana Nissan: Northwestern University, Aligning Science Across Parkinson's (ASAP) Collaborative Research Network, Chevy Chase, MD 20815 Divya.D.A Raj: Northwestern University, Aligning Science Across Parkinson's (ASAP)

Collaborative Research Network, Chevy Chase, MD 20815



Maryana Nissan Northwestern University

DISCLAIMER

DISCLAIMER - FOR INFORMATIONAL PURPOSES ONLY; USE AT YOUR OWN RISK

The protocol content here is for informational purposes only and does not constitute legal, medical, clinical, or safety advice, or otherwise; content added to <u>protocols.io</u> is not peer reviewed and may not have undergone a formal approval of any kind. Information presented in this protocol should not substitute for independent professional judgment, advice, diagnosis, or treatment. Any action you take or refrain from taking using or relying upon the information presented here is strictly at your own risk. You agree that neither the Company nor any of the authors, contributors, administrators, or anyone else associated with <u>protocols.io</u>, can be held responsible for your use of the information contained in or linked to this protocol or any of our Sites/Apps and Services.

## ABSTRACT

This protocol describes the process of Paraformaldehyde fixation [PFA-fixation] and immunofluorescent-labeling of cultured cells.

2	Fixate cells in 4% PFA for 15 minutes at room temperature [RT].
3	Wash cells with 1X PBS twice, for 5 minutes each. Here, cells can be stored in 1X PBS at 4C for up to a week.
4	Permeabilize cells with 1x PBS + 0.1% TriotonX-100 for 5 minutes at RT.
5	Wash with 1X PBS twice, for 5 minutes each wash at RT.
6	Block with 10% serum (from species of secondary) for 1 hour at RT.
7	Drain and incubate with primary antibody diluted in 5% serum overnight at 4C.
8	Wash in 1X PBS thrice, 5 minutes each wash.
9	Detect primary antibody with Alexa Fluor secondary antibody diluted 1:400 in 5% serum at RT for 1 hour in the dark.

- 10 Wash in 1X PBS thrice, for 5 minutes each wash at RT.
- 11 Add mounting media with DAPI and apply the coverslip.
- 12 Store slides in the dark at 4C.