iPSCs

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IMMUNOCYTOCHEMISTRY OF i^{3}NEURONS (Support Protocol 2)

In 1 collection

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1 Works for me dx.doi.org/10.17504/protocols.io.5wtg7en

Neurodegeneration Method Development Community

ABSTRACT

Staining i^{3}Neurons for immunofluorescence (IF) studies is difficult due to the delicate and interconnected nature of the neuronal processes. These processes are easily disrupted in the extensive series of washes in an IF study, and initial dissociation of even a few processes often results in entire sheets of neurons lifting off the culture surface. The best ways to minimize these events are by reducing the number of total washes and by carrying out washes slowly on a tilted dish. Additionally, if possible, IF studies should only be done in neurons 10 days old or younger. After this point, neurons tend to be very delicate and even extremely gentle washing typically causes substantial cell washout.

EXTERNAL LINK

https://doi.org/10.1002/cpcb.51

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Fernandopulle, M. S., Prestil, R., Grunseich, C., Wang, C., Gan, L., & Ward, M. E. (2018). Transcription-factor mediated differentiation of human iPSCs into neurons. Current Protocols in Cell Biology, e51. doi:https://doi.org/10.1002/cpcb.51

fernandopulle2018.pdf

MATERIALS TEXT

- Fresh 16% paraformaldehyde (PFA) solution (Electron Microscopy Sciences, cat. no. 15710)

Paraformaldehyde, 16% (wt/vol)
by Electron Microscopy Sciences
Catalog #: 15710

- Phosphate-buffered saline (PBS; e.g., Gibco, cat. no. 10010049)

Phosphate-buffered saline (PBS) without calcium or magnesium
by Gibco - Thermo Fischer
Catalog #: 10010049

- 10% (w/v) saponin solution (Acros Organics, cat. no. 419231000)

Saponin, From Quillaja Saponaria Molina Pract.
by Fisher Scientific
Catalog #: AC419231000
CAS Number: 74499-23-3

- Donkey serum (Sigma, cat. no. D9663))

Donkey serum
by Sigma Aldrich
Catalog #: D9663

- 96-well plate with neurons to be fixed and stained (see protocols above)
- Antibodies (primary and secondary)
- Sodium azide (Sigma, cat. no. S8032)

Sodium azide
by Sigma Aldrich
Catalog #: S8032

- Hoechst/DAPI dye (20 mg/ml; 10,000×)
- Sterile filters (SteriFlip, Millipore)

- Liquid reagent reservoirs (Thermo, cat. no. 8096-11)

Reagent Reservoirs
Reagent Reservoirs
Thermo Scientific™ 8096-11

- P200 8-channel pipet (for processing 96-well dishes)
- Plate rocker
- Imaging system

SAFETY WARNINGS

Please see SDS (Safety Data Sheet) for hazards and safety warnings.

BEFORE STARTING

NOTE: The following protocol assumes that the experimenter is using a full 96-well dish with the recommended cell counts and medium volumes as indicated in Basic Protocols 6 and 8. Staining on other surfaces (i.e., 8-well chamber slides) may be performed by scaling volumes appropriately.

1 Make **15 ml** of 8 % PFA solution (**7.5 ml** PBS, **7.5 ml** 16 % PFA).

2 Make **50 ml** of antibody blocking solution (3 % donkey serum and 0.1 % saponin in PBS). Filter sterilize.

Detergents other than saponin (i.e., Triton X-100 or Tween-20 at 0.25 %) may also be used. Concentrations should be optimized by the user.

3 Pour the 8 % PFA solution into a liquid reagent reservoir.

4 Retrieve 96-well plate (45,000 to 50,000 cells/100 µl medium in each well) and slowly add **100 µl** 8 % PFA solution to each well with 8-channel pipet. **Do not pipet up and down to mix.**

4 % PFA is a typical fixative for ICC applications. This concentration is used here, but an aspiration step is eliminated by adding an equal volume of 8 %PFA directly to the culture medium on the cells. Pipetting up and down eliminates this advantage by promoting cell dissociation from the culture surface.

5 Incubate at **Room temperature** for **00:10:00** .

Longer fixation times and/or cold incubations may be used as per requirements for particular antibodies.

6 Tilt dish to one side and lower 8-channel micropipet tips so that they contact the wall of each well.
Slowly aspirate the PFA, leaving a small amount (if necessary) at the wall-culture surface interface.

To prevent drying, aspirate only one column of wells at a time. Dispense PFA into a waste liquid reagent reservoir.

7 With plate tilted, slowly dispense **200 µl** PBS onto the same wall in each well, taking care to direct the micropipet tip toward the wall and NOT the culture surface. Liquid should flow smoothly onto culture surface.

PBS without detergent tends to adhere to the wall and then suddenly rush onto the culture surface all at once, promoting cell dissociation. To ensure more gradual flow, gently rub the micropipet tip against the wall in a side-to-side motion while dispensing. This action disrupts the surface tension of the dispensed PBS droplet, providing a mechanical substitute for detergent.

8 Repeat steps 6 and 7 for each column of wells.

go to step #6

9 Once fixative solution has been replaced with PBS, gently rock for 00:05:00 .

10 Repeat steps 6 to 9 two times, each with 100 µl washes of PBS.

go to step #6

11 Aspirate PBS (using plate tilt method).

12 Add 100 µl of blocking solution to each well.

13 Gently rock at Room temperature for 00:30:00 .

14 During blocking, make up primary antibody solution(s) in blocking buffer or 3 % BSA solution.

15 Aspirate blocking solution.

16 Add primary antibody solution to plate (following procedure in steps 6 and 7).

17 Gently rock at Room temperature for 01:00:00 or at 4 °C overnight.

18 Aspirate primary antibody solution.

Primary antibody may be saved and re-used for up to 1 month. Sodium azide (0.02 % final concentration) should be added to any saved antibody solutions to prevent microbial growth.

19 Add 150 µl to 200 µl of blocking solution to wells (following procedure in steps 6 and 7).

20 Gently rock for 00:05:00 .

21 Repeat steps 18 to 20 two times for a total of three washes.

go to step #18

22 Aspirate blocking solution.

23 Add secondary antibody solution (following procedure in steps 6 and 7).

24 Gently rock at **Room temperature** for **01:00:00** .

25 Wash wells with PBS three times (following procedure in steps 6 and 7).

The second-to-last wash can contain Hoechst/DAPI dye if nuclear visualization is desired.

26 Image cells in PBS.

Replace with mounting medium if desired.

27 Store plate at **4 °C** .