MAR 21, 2024

DOI:
dx.doi.org/10.17504/protocols.io.6qpvr8xmolmk/v1

Protocol Citation: Cecilia Tubert, DeNard V Simmons 2024. Immunofluorescence for confocal imaging after slice recording. **protocols.io** https://dx.doi.org/10.17504/protocols.io.6qpvr8xmolmk/v1

MANUSCRIPT CITATION:
https://www.science.org/doi/10.1126/sciadv.abp8701

Immunofluorescence for confocal imaging after slice recording

Forked from Immunofluorescence for confocal imaging

Cecilia Tubert[1], DeNard V Simmons[2]

[1]Northwestern University, Feinberg School of Medicine; [2]Northwestern University

Surmeier Lab

DeNard V Simmons
Northwestern University

ABSTRACT

This protocol describes the steps for immunostaining and confocal imaging of ex vivo slices following an electrophysiological experiment.

ATTACHMENTS

Immunofluorescence for confocal imaging.docx

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

Created: Mar 21, 2024

Last Modified: Mar 21, 2024

PROTOCOL integer ID: 97034

Funders Acknowledgement:
ASAP
Grant ID: 020551

MATERIALS

Immunofluorescence

Materials:

- Pre-cut slices
- Orbital shaker
- 50 ml falcon tubes
- PFA stock solution (recommended: 16% PFA solution, Electron Microscopy Science)
- 10X PBS
- pHmeter and related reagents/tools
- Blocking reagent, e.g.: Normal Goat Serum (NGS) – the correct blocking reagent/blocking solution should be established based on the characteristics of the antibodies used.
- Triton-X100 (detergent)
- Primary antibody(s)
- Secondary antibody(s)
- Hard-drying mounting medium (recommended ProLong Diamond, ThermoFisher Scientific)

- Microscopy slides

- Glass coverslips (recommended #1.5, VWR)

- Liquid PFA waste collection bin

Solutions:

- PBS can be prepared from 10X concentrated solution

- 4% PFA solution is prepared by diluting the concentrated PFA stock and PBS 10X stock in water. For better results, it is recommended to prepare a fresh 4% PFA solution in PBS right before the procedure. Adjust pH of PBS and PFA solutions to 7.3-7.4

Recommended PPE:

- Lab coat/disposable gown

- Examination gloves

Confocal imaging

Materials:

- Pre-mounted microscopy slides

- Confocal laser scanning microscope with appropriate objectives (recommended: 10x/0.4 or a 60x/1.35 immersion) connected to a computer with the appropriate imaging software

- Immersion oil
- Precision wipes
- 70% Ethanol/lens cleaning solution
- Image processing software (recommended: FIJI).

Immunofluorescence - Before the procedure:

1 Prepare PBS

2 Remove slice from the recording chamber. Gently transfer into a plate and bathe with a fixative solution for further processing.

3 Replace fixative with PBS. Slices can be stored in the plate with PBS at 4C. It is recommended to wrap cell plate and its lid with parafilm to avoid PBS evaporation.

4 For longer storage, a preservative (e.q. sodium azide) can be added to the PBS.

5 If needed, prepare aliquots of NGS. Store at -20C and thaw immediately before use.

6 If needed, reconstitute/aliquot antibodies and store them according to manufacturer's instructions. Thaw them on ice immediately before use.

7 It is recommended to prepare the other solutions as needed

Procedure:

8 Select the slices of interest and transfer them in a new plate with PBS.

9 For better results when performing an immunostaining targeting an epitope contained in subcellular organelles, we had better results performing a short permeabilization stage with a higher concentration of detergent. This step is however optional.

10 Prepare permeabilization solution: 0.5% Triton in PBS. The desired volume of permeabilization solution depends on the number of slices to process, the size of the wells/volume of solution needed to cover the slices. For a 24 wells plate, we recommend 1 ml/well of solution for permeabilization/PBS wash/blocking steps, but this can be decreased to ~300ul/well for incubation with antibodies.

11 Remove PBS from each well containing a slice and substitute with permeabilization solution.

12 Incubate for 15 mins with constant gentle movement over orbital shaker.

13 While the slices are incubating with permeabilizing solution, prepare blocking solution (e.g.: 10% NGS, 0.25% Triton-X100 in PBS). This is the solution where the primary antibody/antibodies will be diluted, so when calculating the required volume account for the volume required for both steps (e.g.: 1ml + ~300ul per well).

14 After the 15 minutes, remove permeabilization solution, rinse with PBS, and cover slices with blocking solution.

15 Incubate for 30 mins with constant gentle movement over orbital shaker.

16 During the incubation, dilute the primary antibodies in blocking solution according to recommended dilution.

17 After the 30 mins incubation, remove blocking solution and apply diluted primary antibody/antibodies.

18 Incubate over gentle agitation. Depending on the quality of the antibody used, it can be recommended to incubate the slices with the primary antibody solution over-night at 4°C, with gentle agitation.

19 After primary antibody incubation, remove primary solution and cover slices with PBS.

20 Wash for 10 mins over gentle agitation.

21 Repeat wash step for a total of 3 times.

22 During the washes, prepare the blocking solution for the secondary antibody/antibodies (e.g.: 10% NGS in PBS). Calculate needed volume based on the number of slices/size of wells and dilute secondary antibody/antibodies according to manufacturer's instructions.

23 After the PBS washes are completed, remove PBS and cover slices with the secondary antibody solution.

24 Incubate at room temperature with constant gentle agitation on orbital shaker for 60 mins.

25 After incubation, discard secondary antibody solution and cover with PBS.

26 Repeat 3 10 mins PBS washes as above.

27 After PBS washes, with a fine brush carefully mount slices on clean and labelled microscopy slides.

28 Gently remove excess solution.

29 Let dry in the dark (recommended: overnight).

30 Following day/when the slices have dried on the slide: apply a small amount of hard-drying mounting medium sufficient to cover the slices. Carefully avoid the formation of air bubbles. Gently apply a coverslip over the slices and the mounting medium

31 Let cure overnight in the dark.

After the procedure:

32 Dispose of waste and excess reagents/solution according to institutional guidelines.

33 Clean tools/working station.

34 Once the mounting medium is cured, slides are ready for observation.

35 Fully mounted microscopy slides should be stored in a designated container at 4C until time of observation.

Confocal imaging - Procedure:

36 Turn on laser/microscope/computer according to specific manuals/instructions

37 Access imaging software

38 Load microscopy slides in the designated stage

39 Proceed with observation/image acquisition according to the specific microscope/software manuals

After the procedure:

40 Clean immersion objectives with a lens wipe and the appropriate cleaning solution

41 Export/save images

42 Turn off software/microscope/laser according to specific instructions

43 Images can be observed and appropriately adjusted using an imaging software (e.g. FIJI).

44 Copies of the original unprocessed images acquired should be stored.