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Immunofluorescence for confocal imaging after slice recording ✓ Forked from Immunofluorescence for confocal imaging

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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

MATERIALS

Materials:

Immunofluorescence

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Pre-cut slices

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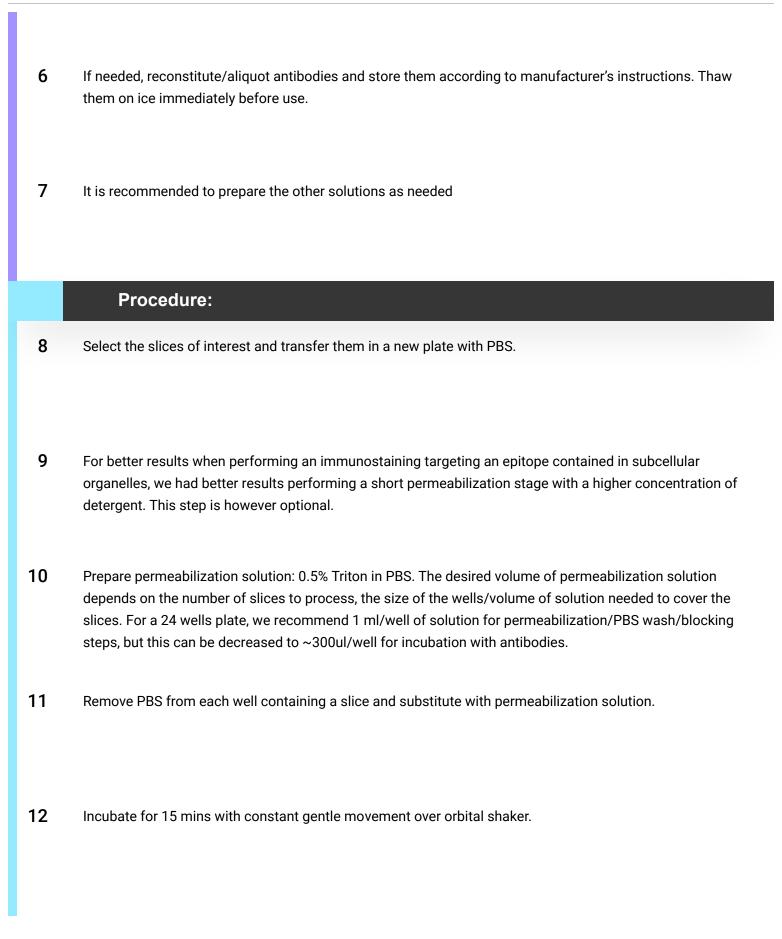
- Orbital shaker
- 50 ml falcon tubes
- PFA stock solution (recommended: 16% PFA solution, Electron Microscopy Science)
- 10X PBS
- pHmeter and related reagents/tools
- Blocking reagent, e.g.: Normal Goat Serum (NGS) the correct blocking reagent/blocking solution should be established based on the characteristics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

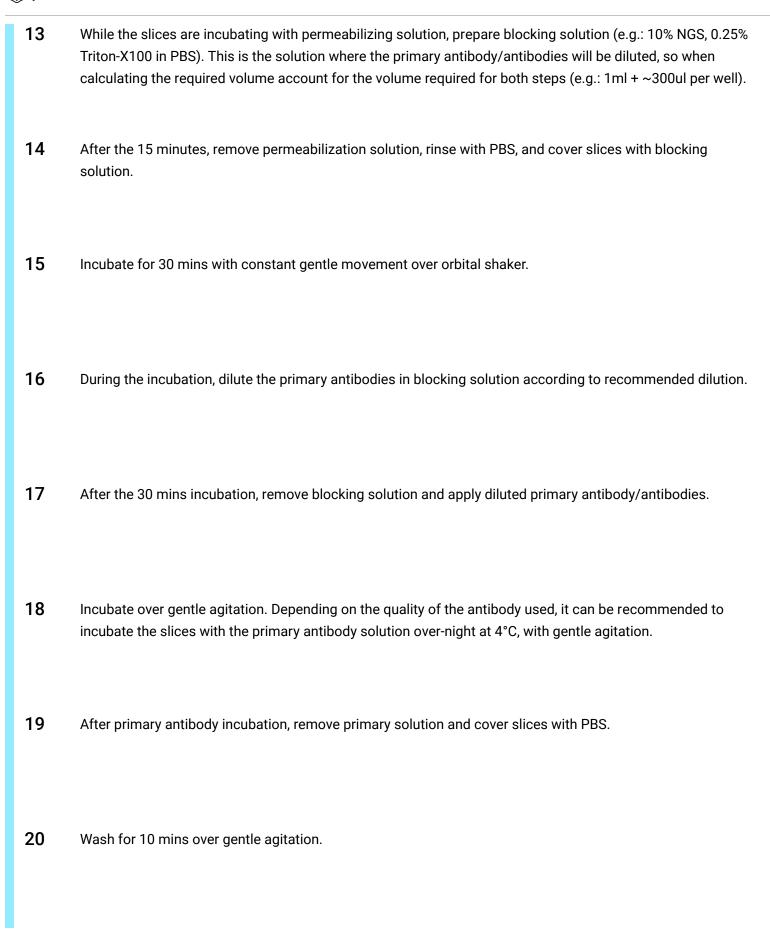
- Microscopy slides
- Glass coverslips (recommended #1.5, VWR)
- Liquid PFA waste collection bin
Solutions:
- PBS can be prepared from 10X concentrated solution
- 4% PFA solution is prepared by diluting the concentrated PFA stock and PBS 10X stock in water. For better results, it is recommended to prepare 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

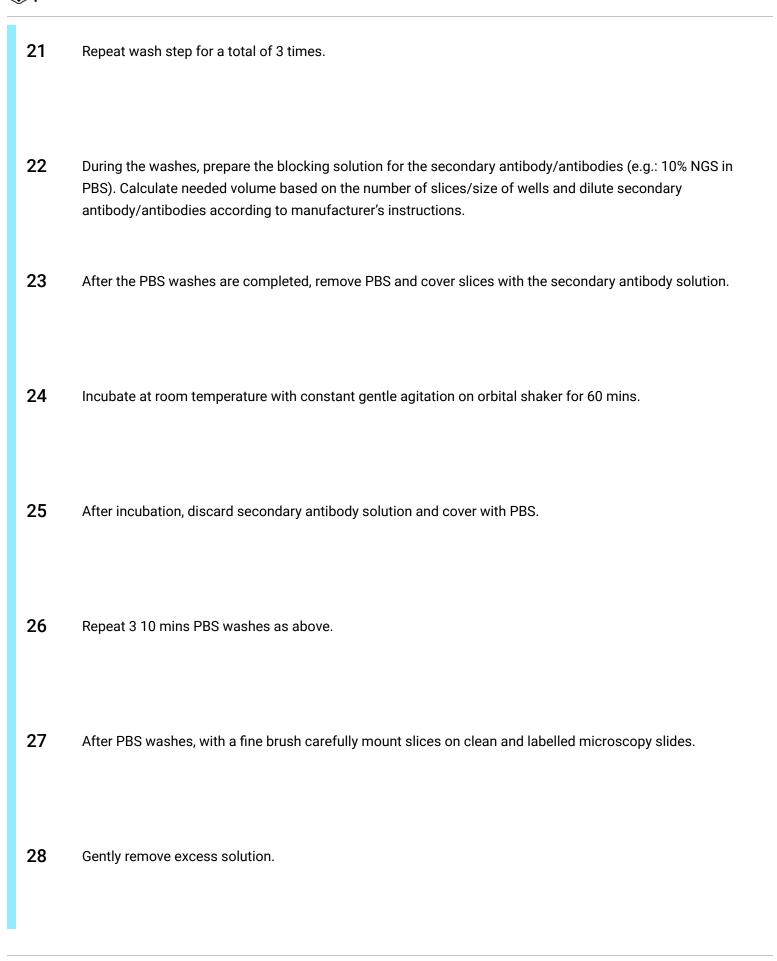
- Immersion oil
- Precision wipes
- 70% Ethanol/lens cleaning solution
- Image processing software (recommended: FIJI).

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.







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.
33	Clean tools/working station. Once the mounting medium is cured, slides are ready for observation.

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Confocal imaging - Procedure:



44

Copies of the original unprocessed images acquired should be stored.