



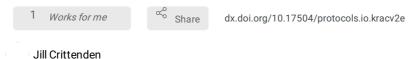
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Immunofluorescence for free-floating brain sections

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ARSTRACT

This protocol describes how to prepare fixed brain sections and process them for immunofluorescence.

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KEYWORDS

fluorescence, immunohistology, free-floating

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SAFETY WARNINGS

Paraformaldehyde (PFA) is toxic, a strong irritant, and flammable as a solid. Prepare and use solutions only under adequate ventilation and with eye and skin protection.

Solution preparation

1 0.4 M NaKPO₄ Phosphate Buffer Stock (dilute this to make 0.1M PB for PFA, mounting solution, and rinse buffer)

112 g K₂HPO₄, (potassium phosphate dibasic; MilliporeSigma cat. # PX1570)

21.2 g Na₂H₂PO₄H₂O (sodium phosphate monobasic; VWR cat. #BDH9298)

2 L Distilled H₂O

0.1 M NaPO₄ Phosphate Buffer Stock (use this to make PBST)

23.1 g Na₂HPO₄ (sodium phosphate dibasic; VWR cat. # 0404)

 $5.04 \text{ g NaH}_2\text{PO}_4\text{H}_2\text{O}$ (sodium phosphate monobasic; VWR cat. # BDH9298)

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PBST working solution

200 ml 0.1M NaPO₄ Phosphate Buffer Stock for PBST

1800 ml Distilled H₂O

17.5 g NaCl sodium chloride, Granular (Macron Fine Chemicals cat. #7532)

0.4 g KCl potassium chloride (Macron Fine Chemicals cat. #6858)

4 ml Triton X-100 (Sigma cat. # T9284)

4% PFA (freshly depolymerized from paraformaldehyde) for perfusion

Prepare in a fume hood, formaldehyde is a biohazard.

Add 4 % w/v paraformaldehyde (Macron Chemicals cat. #2621-59) to 0.1M PB.

Heat mixture to 80-85°C (do not let it go beyond this temperature).

Remove from heat and stir with a stir-bar until all solids have dissolved and the solution is clear.

Allow to cool to room temperature, then filter the solution through filter paper (VWR cat. #28331-081).

If using the same day, keep at room temperature, otherwise, store at 4 °C.

Glycerol cyroprotectant solution

25 ml glycerol (VWR cat. #0854)

75 ml 0.1M PB with 0.1% sodium azide (MP Biomedicals cat. #0210289190)

TSA blocking buffer

Heat 500ml PBST to 50°C

Add 2.5g Blocking Reagent (0.5% final; Perkin Elmer cat. #FP1012)

Stir until dissolved and cool to room temperature.

Use a funnel to filter through paper (VWR cat. #28331-081).

Mounting Solution

Heat 500ml 0.1M PB with 0.1% sodium azide to 55°C

Add 0.4g gelatin (0.08% final; Sigma cat. #G1890)

Cool to room temperature.

Use a funnel to filter through paper (VWR cat. #28331-081).



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Brain tissue preparation

2 Brains are dissected from deeply-anesthetized mice that were trans-cardially perfused with 10 ml of room-temperature 0.9% saline followed by 60 ml of 4% PFA. PFA must be used with appropriate protection and ventilation. Brains are then post-fixed in 4% PFA in glass vials for 2 hours with gentle rocking at 4 °C. Replace PFA solution with glycerol cryoprotectant solution until the brain sinks (overnight), at 4 °C. Store the brains in the same solution at 4 °C until sectioning. For sectioning, remove the brains from storage cryoprotectant solution, freeze in powdered dry ice on a chuck, and cut at 30 μm intervals on a sliding microtome. Put sequential sections into adjacent wells of a multi-well plastic tray containing 0.1% sodium azide in 0.1 M PB for storage at 4 °C until immunostaining.



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Immunofluorescence reactions

- 3 Rinse sections in PBST, 3 x 2 minutes
- 4 Incubate in blocking solution, 20 minutes
- 5 Incubate in primary antibodies diluted in blocking solution, 1 overnight at 4°C
- 6 Incubate in secondary antibodies diluted in blocking solution, 2 hours at room temp. in dark
 [We use Alexa fluorophore conjugated secondary antibodies (Thermo Fisher Scientific), diluted 1:300]
- 7 Rinse in PBST, 3 x 2 min
- Rinse in 0.1M PB, 3 x 2 minutes
- 9 Submerge sections in mounting solution in a large glass Petri dish and use a paintbrush to move them onto glass microslides (VWR #48311-703)
- 10 Let sections sit flat, covered loosely by foil, until they appear almost dry, 15 60 minutes, depending on the room humidity
- 11 Coverslip with ProLong™ Gold Antifade Mountant with DAPI (Thermo Fisher Scientific cat. #P36931)
- 12 Store slides in the dark at 4 0 C