Jun 28, 2021

Immunofluorescence for free-floating brain sections

Jill R. Crittenden[1], Tomoko Yoshida[1], Margaret I. Davis[2], Ann M. Graybiel[1]

[1]McGovern Institute for Brain Research, Massachusetts Institute of Technology, Cambridge, MA, 02138, USA;

[2]Laboratory for Integrative Neuroscience, National Institute on Alcohol Abuse and Alcoholism, National Institutes of Health, USA

dx.doi.org/10.17504/protocols.io.kracv2e

Jill Crittenden

ABSTRACT

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

DOI

dx.doi.org/10.17504/protocols.io.kracv2e

PROTOCOL CITATION

Jill R. Crittenden, Tomoko Yoshida, Margaret I. Davis, Ann M. Graybiel 2021. Immunofluorescence for free-floating brain sections. **protocols.io** https://dx.doi.org/10.17504/protocols.io.kracv2e

KEYWORDS

fluorescence, immunohistology, free-floating

CREATED

Nov 09, 2017

LAST MODIFIED

Jun 28, 2021

PROTOCOL INTEGER ID

8706

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 $NaKPO_4$ Phosphate Buffer Stock (dilute this to make 0.1M PB for PFA, mounting solution, and rinse buffer)**

112 g K_2HPO_4, (potassium phosphate dibasic; MilliporeSigma cat. # PX1570)

21.2 g $Na_2H_2PO_4H_2O$ (sodium phosphate monobasic; VWR cat. #BDH9298)

2 L Distilled H_2O

0.1 M $NaPO_4$ Phosphate Buffer Stock (use this to make PBST)

23.1 g Na_2HPO_4 (sodium phosphate dibasic;VWR cat. # 0404)

5.04 g $NaH_2PO_4H_2O$ (sodium phosphate monobasic;VWR cat. # BDH9298)

PBST working solution

200 ml 0.1M $NaPO_4$ Phosphate Buffer Stock for PBST
1800 ml Distilled H_2O
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 80-85ºC (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 ºC.

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).

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.
https://www.sigmaaldrich.com/MSDS/MSDS/DisplayMSDSPage.do?country=US&language=en&productNumber=158127&brand=SIAL&PageToGoToURL=https%3A%2F%2Fwww.sigmaaldrich.com%2Fcatalog%2Fproduct%2Fsial%2F158127%3Flang%3Den

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.

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.
https://www.sigmaaldrich.com/MSDS/MSDS/DisplayMSDSPage.do?country=US&language=en&productNumber=158127&brand=SIAL&PageToGoToURL=https%3A%2F%2Fwww.sigmaaldrich.com%2Fcatalog%2Fproduct%2Fsial%2F158127%3Flang%3Den

Citation: Jill R. Crittenden, Tomoko Yoshida, Margaret I. Davis, Ann M. Graybiel (06/28/2021). Immunofluorescence for free-floating brain sections. https://dx.doi.org/10.17504/protocols.io.kracv2e

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^{o}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

8 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

Citation: Jill R. Crittenden, Tomoko Yoshida, Margaret I. Davis, Ann M. Graybiel (06/28/2021). Immunofluorescence for free-floating brain sections. https://dx.doi.org/10.17504/protocols.io.kracv2e