

Immunofluorescence Staining on Mouse Embryonic Brain Sections

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[Abstract] This protocol comprises the entire process of immunofluorescence staining on mouse embryonic brains, starting from tissue preparation to mounting of the tissue sections.

Materials and Reagents

1. Paraformaldehyde (PFA)

It can be ordered as 16% PFA from Electron Microscopy Sciences (catalog number: 15710), and diluted to 4% before use. However, we usually make the 4% solution from the powder (Sigma-Aldrich, catalog number: 158127). Here is how to make 4% PFA:

- a. In a 500 flask, weigh 4 g PFA in 80 ml PBS.
- b. Stir and heat the mixture on a magnetic heater. Keep the temperature below 60 °C during the entire process to avoid breakdown of the polymers.
- c. When temperature is close to 60 °C, add a few drops of 10 N sodium hydroxide. The mixture will gradually become clear.
- d. Filter the solution into a new flask and cool down to room temperature.
- e. Neutralize with hydrochloric acid to pH 7.
- f. Add PBS to 100 ml.

2. O.C.T. (TFM Tissue Freezing Medium, TFM-5, clear color)

3. Phosphate buffered saline (PBS)

4. Goat serum

5. Triton X-100

6. Secondary antibodies:

Cy2 (or cy3, or cy5)-conjugated goat-anti-mouse (or rabbit) IgG.

(Jackson ImmunoResearch, F (ab) 2 fragments of affinity-purified antibody)

7. Hoechst33258 (Sigma-Aldrich, catalog number: 861405)

8. Antifade reagent (Life Technologies, Invitrogen™, catalog number: P36930)

9. Ethanol

10. 4 N hydrochloric acid

11. Sodium hydroxide
12. Blocking buffer (see Recipes)

Equipment

1. Plastic cryomolds (Ted Pella, catalog number: 27110)
2. Cryostat sectioning machine
3. SuperFrost Plus slides (VWR International, catalog number: 48311-703)
4. Dark humidified chamber: Made from a square petri dish containing kimwipes that are soaked in water. Wrap the chamber with aluminum foil to protect the slides from light.
5. PAP pen
6. 500 flask
7. Ice bucket
8. Magnetic heater

Procedure

1. Perfuse embryos (E15-19) with 4% paraformaldehyde. Dissect out the brains and postfix in 4% paraformaldehyde overnight.
2. In plastic cryomolds, embed brains in O.C.T., with the olfactory bulb facing downward.
3. In an ice bucket, carefully drop some dry ice into 100 ml ethanol. When the bubbles stop popping, carefully transfer the mold into the dry ice-cooled ethanol. Watch the orientation of the brain before the O.C.T. freezes. Avoid splashing of ethanol into the mold. When the O.C.T. blocks completely freeze, store the block in -20 °C. The tissues are OK at -20 °C for two months, but best staining results are obtained on freshly frozen tissues.
4. Section brains in cryostat sectioning machine, 10-20 µm thick. Attach brains to SuperFrost Plus slides. Air dry the sections for 20 min. The sections can be stored at -20 °C for two months, but best staining results are obtained on freshly cut tissues. Perform the following staining procedure in a dark humidified chamber.
5. Wash with PBS, 3 times to clear out of the O.C.T. (*Optional:* For BrdU staining, brain sections were incubated in 4 N hydrochloric acid for 2 h at room temperature (RT) to unmask the antigen, followed by three washes in PBS).
6. Slightly dry the slides, but make sure the tissue sections are kept wet. Use PAP pen to circle the four edges of the slides so that the staining solutions are confined in the circle.
7. Add blocking buffer to the slides. Make sure that all tissue sections are covered with blocking buffer. Incubate at RT, 1 h.

8. Aspirate blocking buffer, add primary antibody diluted in blocking buffer. Incubate at RT, 1 h (Or 4 °C, overnight). If more than one antibody is used in the staining, can add them simultaneously.
9. Wash with PBS, 3 times, 5 min each.
10. Add 2nd antibody diluted in blocking buffer, Incubate at RT, 1 h. If more than one antibody are used in the staining, can add the 2nd antibodies simultaneously. **Don't overstain with 2nd antibody!!**
11. Wash with PBS, 3 times, 5 min each.
12. Stain the nuclei with Hoechst33258 (final concentration 1 µl/ml in PBS), at RT, 10 min.
13. Wash with PBS, 3 times, 5 min each.
14. Mount in antifade reagent.

Recipes

1. Blocking buffer
0.2% triton X-100 in PBS
2% goat serum (the blocking serum should be from the same animal where the second antibody is produce)

Acknowledgments

This protocol was adapted from and used in Ge *et al.* (2009) and Mao *et al.* (2010).

References

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