Anti-Alpha II Spectrin Immunohistofluorescence Protocol

Catalog #: 98-A2SM
Species: mouse
Tissue: Rat Cerebellum

Fixation: 4% Paraformaldehyde in 1X PBS for 2 hours
Antibody incubation: Primary Antibody- 4C, overnight
Secondary Antibody- RT, 2 hours or 4C, overnight
Antigen Retrival: None

Materials Required

- ✓ **Fixative:** 4% Paraformaldehyde in freshly prepared 1X PBS
- ✓ **isopentane:** chilled to -160C
- ✓ **acetone:** chilled to -20C
- ✓ **1X PBS:** 137 mM NaCl, 28 mM Na_2HPO_4, 5.4 mM KCl, 2.9 mM KH_2PO_4 , pH 7.6
- ✓ **20% /30% sucrose buffer:** 20/30g of sucrose in 100mls of 1xPBS
- ✓ **Blocking buffer:** 1X PBS with 1% goat serum, also use for secondary incubation buffer
- ✓ **Incubation buffer:** 1X PBS with 0.1% goat serum
- ✓ **Secondary Antibody:** example used is Goat-Anti-Mouse Alexa 488 from ThermoFisher
- ✓ **Mounting media:** Vector Laboratories Vectashield with DAPI Cat #: H-1200

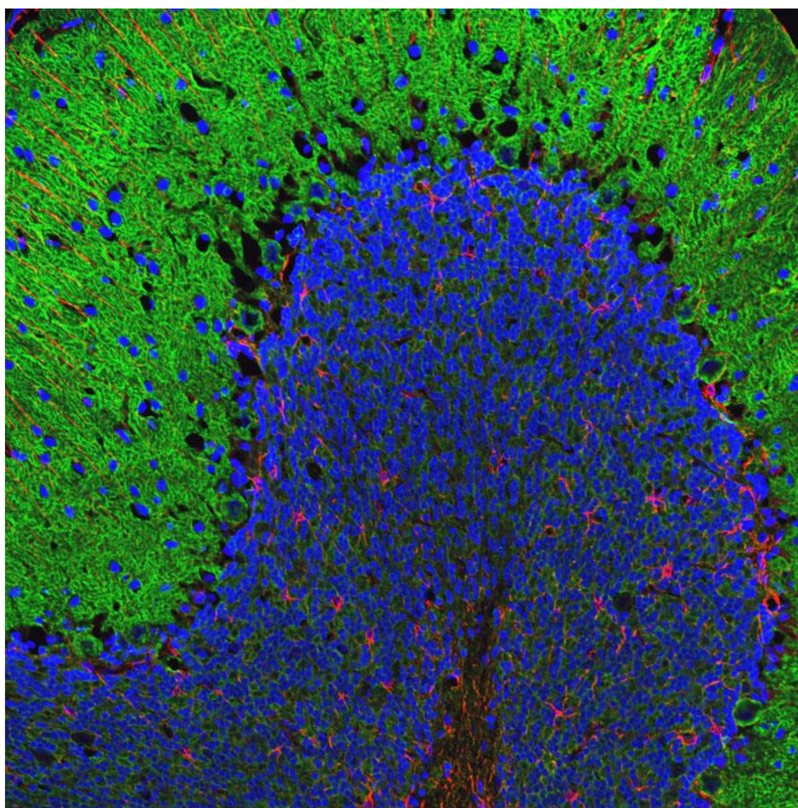
Before you begin

This protocol can be used for tissues fixed with or without perfusion. If tissues are harvested without perfusion, slice tissues into 0.5cm sections and place in 4% paraformaldehyde for 2 hours at RT. Submerge sections into a sufficient volume of fixative, for proper fixation a recommended minimum volume of 20x. Additionally each section of tissue should be in a separate container. If tissues are harvested after perfusion, harvest and place in fixative. For optimal antibody epitope binding, tissues should not stay longer than 2 hours in fixative.

Protocol

1. Place fixed tissue section into 20% sucrose in 1X PBS overnight at 4C.
2. Transfer tissue section into 30% sucrose in 1X PBS for 2-3 days at 4C.
   Tech Tip:
   a. To prevent ice crystals from forming on tissue and destroying antibody epitope binding sites, do not remove the tissue until it has sunk to the bottom of the beaker to ensure complete sucrose infiltration.
3. Transfer tissue into isopentane that is at -160C. This can be done by cooling the isopentane in a plastic beaker placed in liquid nitrogen. Once tissue is frozen remove and immediately section with cryostat or store at -70C.

4. Mount tissue onto cryostat and cut tissue into 45 mu thick sections at -25C. Place sections into PBS in a container to float freely.
5. Rinse tissue sections with PBS 3 times, in 5 minute intervals.
6. Block tissue sections with blocking buffer for 1 hour at RT.
7. Rinse tissue sections with PBS 3 times, in 5 minute intervals.
8. Dilute Anti-alpha-II-spectrin (Cat. # 98-A2SM) to 1:2000 in incubation buffer. Incubate sections for 2 hours at room temperature or overnight at 4C.
9. Rinse tissue sections with PBS 3 times, in 5 minute intervals.
10. Dilute secondary antibody in incubation buffer per manufacturer's recommendation. Incubate tissue sections for 1 hour at room temperature or overnight at 4C.
    Tech Tip:
    a. Alexa Fluor 488 dye diluted 1:2000 was used to produce the image below.
11. Remove secondary antibody and wash the tissue section with PBS 3 times, in 5 minute intervals.
12. Place tissue sections onto slide and apply mounting medium. Gently place glass cover slip before viewing under the microscope.
    Tech Tip:
    a. Any mounting media can be used, for this protocol Vector Laboratories Vectrashield medium was used. Cat #: H-1200.



Immunofluorescence of a section of rat cerebellum selectively labeling the submembraneous cytoskeleton on neurons and cell bodies and dendrites of Purkinje cells with alpha-II-spectrin (cat #98-A2SM, 1:2000, green) and labels the processes of Bergmann glia and astrocytes with anti-GFAP (catalog #621-GFAP, 1:5000,red).