Anti-NMDA Receptor, NR1 Subunit Antibody Immunohistofluorescence Protocol

Catalog #: 1508-NR1
Species: mouse
Tissue: Rat cortices

Fixation: 4% Paraformaldehyde/ 4% sucrose in 1X PBS (intracardial perfusion, 1 hour)
Antibody incubation: Primary Antibody- 4C, overnight Secondary Antibody- RT, 1 hour

Materials Required

- ✓ **Fixative:** 4% Paraformaldehyde/ 4% sucrose in freshly prepared 1X PBS
- ✓ **OCT compound:** Fisher Healthcare Cat #: 23-730-571
- ✓ **1X PBS:** 137 mM NaCl, 28 mM Na_2HPO_4, 5.4 mM KCl, 2.9 mM KH_2PO_4 , pH 7.6
- ✓ **30% sucrose buffer:** 30g of sucrose in 100mls of 1xPBS
- ✓ **Blocking buffer:** 1X PBS with 1% BSA
- ✓ **Primary Incubation buffer:** 1X PBS with 10% goat serum, 0.5% Triton X-100
- ✓ **Secondary Incubation buffer:** 1X PBS with 10% goat serum, 0.5% Triton X-100
- ✓ **Secondary Antibody:** example used is goat anti-rabbit IgG fluorescein isothiocyanate (FITC) from Jackson Immuno Labs
- ✓ **Mounting media:** ProLong Gold Antifade Mountant Medium 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. Make sure the slices have sufficient volume of fixative for proper fixation. If tissues are harvested after perfusion, harvest and place in fixative for 15-30 minutes. For optimal antibody epitope binding, tissues should not be stored in fixative. It is best to store fixed tissue in cryoprotectant solution of 25% sucrose buffer.

Protocol

1. Place fixed tissue specimen into 25% 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.
2. Transfer tissue into OCT compound and freeze at -80C overnight.
3. Mount tissue onto cryostat and cut tissue into 12-16 micron thick sections at -25C. Mount tissue sections onto gelatin coated slides.
   Tech Tip:
   a. Slides can be stored at -20C for long term storage.
4. Thaw sections for 10 minutes at RT.

5. Wash the sections 3 times with blocking buffer for 10 minutes.
6. Block slides with blocking buffer for 1 hour at RT.
7. Wash the slides 3 times with 1X PBS for 10 minutes.
8. Dilute Anti-$GABA_A$ Transporter (GAT) 2 Antibody (Cat. # 881-GAT2) to 1:100 in primary incubation buffer. Incubate sections overnight at 4C.
9. Wash the slides 3 times with 1X PBS for 10 minutes.
10. Dilute secondary antibody in secondary incubation buffer per manufacturer's recommendation. Incubate sections for 2 hours at room temperature.

    Tech Tip:

    a. FITC dye diluted 1:50 was used to produce the image.
11. Remove secondary antibody and wash with 1x PBS 3 times, in 10 minute intervals.
12. Apply mounting medium onto slide and gently place glass cover slip before viewing under the microscope.

    Tech Tip:

    a. Any mounting media can be used, for this protocol ProLong Anti-Fade medium was used. Cat #: H-1200.

Reference:

Johnson, J., Chen, T. K., Rickman, D. W., Evans, C., & Brecha, N. C. (1996). Multiple γ-aminobutyric acid plasma membrane transporters (GAT-1, GAT-2, GAT-3) in the rat retina. *The Journal of comparative neurology*, 375(2), 212.