Anti-NMDA Receptor, NR2A Subunit Antibody Immunohistofluorescence Protocol

Catalog #: 1495-NR2A
Species: rabbit
Tissue: Rat brain

Fixation: Transcardial perfusion, 4% Paraformaldehyde in 1X PBS, post fixed overnight
Antibody incubation: Primary Antibody- 4C, overnight
Secondary Antibody- RT, 1 hours or 4C, overnight
Antigen Retrieval: None

Materials Required

- ✓ **Fixative:** 4% Paraformaldehyde in freshly prepared 1X PBS
- ✓ **dry ice**
- ✓ **cryoprotectant solution**
- ✓ **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 10% normal goat serum/ 0.5% Triton X-100
- ✓ **Wash buffer:** 1X PBS with 0.1% Triton X-100
- ✓ **Incubation buffer:** 1X PBS with 5% normal goat serum/ 0.3% Triton X-100/ 1% BSA
- ✓ **Secondary Antibody:** example used is Goat-Anti-Rabbit CY3 from Jackson ImmunoResearch Cat#: 111-165-003
- ✓ **Mounting media:** ProLong™ Gold Antifade Mountant with DAPI Cat #: P36931

Before you begin

The tissue used in this protocol was perfused transcardially. Alternatively, tissues can be fixed without perfusion. Immediately after sacrifice cut tissue into 0.5cm sections and place in 4% paraformaldehyde overnight at 4C. Submerge sections into a sufficient volume of fixative, for proper fixation a recommended minimum volume of 20x each in separate containers. For optimal antibody epitope binding, tissues should not stay longer than 24 hours in fixative.

Protocol

1. Transfer tissue section into 30% sucrose in 1X PBS for 72 hours 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. Freeze tissue sections with dry ice and store at -80C.
3. Mount tissue onto cryostat and cut tissue into 40 mu thick sections at -20C. Place sections into cryoprotectant solution to float freely at -20C.
4. Rinse tissue sections with PBS 3 times, in 5 minute intervals.

5. Block tissue sections with blocking buffer for 1 hour at RT.
6. Dilute Anti-NMDA Receptor, NR2A Subunit (Cat. # 1495-NR2A) to 1:500 in incubation buffer. Remove blocking buffer and add diluted antibody in incubation buffer. Incubate sections overnight at 4C.
7. Rinse tissue sections with wash buffer 3 times, in 10 minute intervals.
8. Dilute secondary antibody in 1X PBS per manufacturer's recommendation. Incubate tissue sections for 1 hour at room temperature.

   Tech Tip:

   a. A goat anti-rabbit- CY3 dye diluted 1:1000 was used for this protocol.
9. Remove secondary antibody and wash the tissue section with 1X PBS for 10 minutes.
10. Place tissue sections onto slide and apply mounting medium. Air dry sections for 30 minutes. Gently place glass cover slip before viewing under the microscope.

    Tech Tip:

    a. Any mounting media can be used, for this protocol ProLong™ Gold Antifade Mountant with DAPI was used. Cat #: P36931.

Reference:

Aroniadou-Anderjaska, V., Pidoplichko, V.I., Figueiredo, T.H. and Braga, M.F., 2018. Oscillatory synchronous inhibition in the basolateral amygdala and its primary dependence on NR2A-containing NMDA receptors. *Neuroscience*, 373, pp.145-158.