Anti- NMDA Receptor, NR2B Subunit (N-terminal) Immunocytofluorescence Protocol

Catalog #: 1503-NR2B
Species: rabbit
Tissue/ Cells: Rat cortical striatal cocultures

Fixation: 4% paraformaldehyde, 4% sucrose in 1xPBS, 10 minutes
Antibody incubation: Primary Antibody- 4C, overnight Secondary Antibody- RT, 1.5 hours

Materials Required

- ✓ **Fixative:** 4% paraformaldehyde, 4% sucrose in 1xPBS
- ✓ **1x PBS:** 137 mM NaCl, 28 mM Na_2HPO_4, 5.4 mM KCl, 2.9 mM KH_2PO_4, pH 7.6
- ✓ **Permeabilization (Wash) solution (PBST):** 0.03% Triton-X 100 in 1xPBS
- ✓ **Blocking buffer:** 10% NGS (normal goat serum) in 1xPBS
- ✓ **Incubation buffer:** 2% NGS in PBST
- ✓ **Secondary Antibody:** example used is Goat-Anti-Rabbit Alexa 488 from ThermoFisher
- ✓ **Mounting media:** DAPI Fluoromount-G Cat #: 0100-20
- ✓ **Glass cover slip**

Before you begin

This protocol is intended for cultured neurons that have stimulated synaptic and extra-synaptic NMDARs. For preparing, growing, and propagating these cocultured cells reference Kaufmann et al (2012).

Protocol

1. Draw off culture medium with aspirator and add 1 ml of 4% PFA, 4% sucrose 1xPBS fixative solution to the coverslip. Incubate at room temperature for 10 minutes.
2. Remove the fixative and wash with 1xPBS 3 times.
3. Permeabilize cover slips with Wash Solution for 5 minutes.
4. Remove wash solution and add blocking buffer. Incubate for 30 minutes at room temperature.
5. Rinse coverslips with PBS 3 times, in 10 minute intervals.
6. Dilute Anti-NMDA Receptor, NR2B Subunit, N-terminal (Cat. # 1503-NR2B) to 1:400 in incubation buffer. Incubate cells overnight at 4C.
7. Remove primary antibody and wash with PBST 3 times, in 10 minute intervals.
8. Dilute secondary antibody in incubation buffer per manufacturer's recommendation. Incubate cells for 1.5 hours at room temperature.
   Tech Tip:
   a. Alexa Fluor 488 dye diluted 1:2000 was used in this protocol.
9. Remove secondary antibody and wash with PBST 3 times, in 5 minute intervals.

10. Apply mounting medium intended for fluorescence onto dish and gently place glass cover slip before viewing under the microscope.

    Tech Tip:

    a. There are various mounting medias for fluorescence that can be used, for this protocol the medium used was from Southern Biotech, DAPI Fluoromount-G Cat #: 0100-20.

Reference:

Kaufman, A.M., Milnerwood, A.J., Sepers, M.D., Coquinco, A., She, K., Wang, L., Lee, H., Craig, A.M., Cynader, M. and Raymond, L.A., 2012. Opposing roles of synaptic and extrasynaptic NMDA receptor signaling in cocultured striatal and cortical neurons. *Journal of Neuroscience*, 32(12), pp.3992-4003.