Protocol for GluN1 Antibody (Cat. No. 114 011)
Immunocytochemistry (ICC)
Fluorescence Staining - Cell Surface Epitopes

This protocol is suitable for the immunocytochemical (ICC) analysis of extracellular protein domains localizing on the cell-surface. Usualy, the primary antibody can be incubated in the culture medium itself or in other suitable physiological buffers like Krebs-Ringer solution.
The surface bound antibodies can be internalized by endocytosis over time or after stimulation. This effect can be minimized by carrying out the incubation and first washing steps on ice with pre-cooled solutions. After fixation, all steps can be carried out at room temperature (RT)

Materials and reagents

- **Cell incubation solution:** Culture medium or physiological buffer/solution suitable for the cells to be examined
- **PBS:** Phosphate buffered saline (200 mM NaCl, 2.5 mM KCl, 8 mM Na_2HPO_4, 1.5 mM KH_2PO_4), pH 7.4
- **Fixation solution**: 4% FA (4% PFA), 4% sucrose in PBS, pH 7.4
- **Blocking buffer:** 10% normal serum, 0.1% Triton X-100 in PBS (normal serum from the host-species of the secondary antibodies is recommended)
- **Incubation buffer:** 5% normal serum, 0.1% Triton X-100 in PBS (normal serum from the host-species of the secondary antibodies is recommended)
- **Primary antibody:** GluN1 antibody (cat. no. 114 011)
- **Secondary detection reagent:** Fluorophore conjugated mouse specific secondary antibody.
- **Mounting medium**
- *Optional: DAPI nuclear stain*

Procedure

1. Incubate cells in **cell incubation buffer** containing the primary antibody at a **dilution of 1:1000** for up to 30 min at 37°C or on ice.

2. Wash cells briefly with **PBS** two times.

3. Fix cells with F**ixation solution** for 15 min at RT.

4. Wash three times with **PBS** for 10 min each.

5. Incubate for 30 min with **blocking buffer**.

6. Wash three times with **PBS** for 10 min.

7. Incubate in **incubation buffer** containing the fluorophore conjugated secondary antibody (**secondary detection reagent**) for 1 h at RT diluted according to the manufacturers recommendations.
    - *Optional: Add DAPI to the secondary antibody solution.*
    - *Notes:*
        - *Avoid bright light when working with the secondary antibody to minimize photo bleaching of the fluorescent dye.*

- *In multiplex staining, make sure to use secondary antibodies cross-adsorbed against the host species of the other primary antibody used in your experiment. Ideally, all secondary antibodies should come from the same host species. If not, make sure that they have been cross-adsorbed against IgGs of the host-species of the other secondary antibody as well. This avoids cross-reaction between the secondary antibodies.*

8. Wash three times with **PBS** for 10 min.

9. Mount coverslips.

Note: This protocol has been validated in the SYSY Antibodies laboratories to ensure consistent and reliable staining results. However, for achieving the best specific signal with minimal background, the optimal antibody concentration, incubation temperature, and incubation duration should be optimized for each experiment.