Protocol for GluA Antibody (Cat. No. 182 411AT565) 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:** Fluorescent labeled GluA antibody (cat. no. 182 411AT565)
- **Mounting medium**
- *Optional: DAPI nuclear stain*

Procedure

1. Incubate cells in **cell incubation buffer** containing the primary antibody at a **dilution of 1:100 to 1:500** 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.
   - The primary antibody carries a fluorophore. No secondary antibody is required. *Note: Avoid bright light when working with fluorescently labeled primary antibodies to minimize photo bleaching of the fluorescent dye.*

7. 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.