SYSY Antibodies

Tailor-made Antibodies
and Tools for Life Science

Protocol for VGAT Antibody (Cat. No. 131 103)
Immunocytochemistry (ICC)
Fluorescence Staining - Recycling Synaptic Vesicles

This protocol is suitable for the immunocytochemical analysis of actively recycling synaptic vesicles. During neurotransmitter release, the synaptic vesicles fuse with the pre-synaptic membrane, so that the inner vesicle membrane is transiently exposed to the extracellular space. In cultured primary neurons, luminal protein epitopes can thus be bound by antibodies added to the culture medium or a suitable physiological buffer like Krebs-Ringer solution, which are then incorporated into the synaptic vesicles during spontaneous or stimulated clathrin-mediated endocytosis.
With this approach, active synapses with ongoing vesicle recycling can be selectively labelled. For more information, take a look at our featured topic: Labeling of recycling synaptic vesicles.

Materials and reagents

- **Cell incubation solution:** Culture medium or Krebs-Ringer-HEPES solution.
  - Krebs-Ringer HEPES solution: (25mM HEPES, 128 mM NaCl, 4,8 mM KCl, 1,3 mM CaCl, 1,2 mM $NgSO_4$, 1,2 mM KH_2/K_2HPO_4, 5,6% glucose), pH 7.4
- **Stimulation solution**: (25mM HEPES, 75,8 mM NaCl, 57 mM KCl, 1,3 mM CaCl, 1,2 mM $NgSO_4$, 1,2 mM KH_2/K_2HPO_4, 5,6% glucose), pH 7.4)
- **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) in PBS, 4% sucrose, pH 7.4
- **Primary antibody:** VGAT antibody (cat. no. 131 103)
- **Secondary antibody:** Fluorophore conjugated rabbit specific secondary antibody
- *Optional: DAPI nuclear stain*

Procedure

1. To label spontaneously recycling synaptic vesicels, incubate cells in **cell incubation solution** containing the primary antibody at a **dilution of 1:100 to 1:200** for up to 30 min at 37°C.

   Alternative: To label recycling synaptic vesicels after stimulation, incubate cells in **stimulation solution** containing the primary antibody at a **dilution of 1:100 to 1:200** for 5 min at 37°C.

2. Wash cells briefly with **PBS** or **Cell icubation solution** according to your experimental setup.

3. Fix cells with **Fixation 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. Incubate in **incubation buffer** containing the **secondary antibody** 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.*

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

8. 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.