SYSY Antibodies

Tailor-made Antibodies
and Tools for Life Science

# Protocol for VGAT Antibody (Cat. No. 131 103C5)
# 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 (optional)
- **Blocking buffer:** 10% normal serum, 0.1% Triton X-100 in PBS (if secondary antibodies are used normal serum from the host-species of the secondary antibodies is recommended for blocking)
- **Incubation buffer:** 5% normal serum, 0.1% Triton X-100 in PBS (if secondary antibodies are used normal serum from the host-species of the secondary antibodies is recommended)
- **Primary antibody:** Fluorescent labeled VGAT antibody (cat. no. 131 103C5)
- **Mounting medium**
- *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. The staining can be analyzed immediatly. Subsequent fixation and further processing like incubation with additional primary antibodies for multiplexing is possible. Refer to the antibody specific protocols to check compatibilty.

4. *Optional: Fix cells with Fixation solution for 15 min at RT and proceed with additional primary and secondary antibody*

*incubation steps according to the antibody specific protocol.*
*Optional: Add DAPI to the secondary antibody solution.*

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

6. 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.*