SYSY Antibodies

Tailor-made Antibodies
and Tools for Life Science

Protocol for VGAT Antibody (Cat. No. 131 103CpH)
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.

CypHer5E is a pH sensitive dye fluorescent only at acidic pH. VGAT Antibody (Cat. No. 131 103CpH is intended for live monitoring of actively recycling synaptic vesicles. Compared to AcidiFluor Orange, CypHer5E is more sensitive for photobleaching. Long exposure times should be avoided. However, this property can also be employed to selectively bleach fluorescent dye molecules exposed to acidic pH. Dye molecules still residing on the outer cell membrane at physiological pH will not be bleached and are available for subsequent internalization and analysis.

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
- **Primary antibody:** Fluorescent labeled VGAT antibody (cat. no. 131 103CpH)

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. Immediately analyze the staining while the cells are still alive. According to your experimental design, appropriate conditions to prevent or to stimulate further recycling events have to be applied. CypHer5E is a pH sensitive dye fluorescent only at acidic pH.

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.