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

AcidiFluor Orange is a pH sensitive dye fluorescent only at acidic pH. VGAT Antibody (Cat. No. 131 103AF is intended for live monitoring of actively recycling synaptic vesicles. AcidiFluor Orange is more photostable compared to CypHer5E.

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 103AF)

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 1:300** 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 1:300** 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. AcidiFluor Orange 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.