Reference Protocol for Chil3 / YM1 (HS-442 117) Fluorescence Immunostaining of Free-Floating Vibratome Sections

Tissue Fixation

- PFA perfused and fixed vibratome tissue-sections

Materials and Reagents

- **TBS:** 20 mM Tris, pH 7.2, 150 mM NaCl
- **Blocking buffer:** 10% normal serum, 0.3% Triton X-100 in TBS (normal serum from the host-species of the secondary antibody is recommended for blocking)
- **Incubation buffer:** 5% normal serum, 0.3% Triton X-100 in TBS (normal serum from the host-species of the secondary antibody is recommended for incubation)
- **Secondary antibody:** anti-rat secondary antibody conjugated to fluorescent dye
- Mounting medium
- **Optional:** DAPI (4 mg/ml)

Method

1. Transfer the free-floating sections into a staining dish containing **TBS**.
2. Transfer the sections to the **blocking solution** and block for 1 h at RT (orbital shaker: 70 - 80 rpm).
3. Transfer the sections to the **incubation buffer** with the primary antibody at a **1:2500 dilution** and incubate overnight at 4°C (orbital shaker: 60 rpm).
4. Wash three times for 10 min in **TBS** (RT; orbital shaker: 70 - 80 rpm).
5. Transfer the sections to the **incubation buffer** with the secondary antibody diluted to the manufacturer's recommended concentration and incubate for 1 h at RT (orbital shaker: 70-80 rpm).

Note: *Avoid bright light when working with the secondary antibody to minimize photo bleaching of the fluorescent dye. In Multiplex stainings 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.*

6. Wash three times for 10 min in **TBS** (RT; orbital shaker: 70-80 rpm).
   Optional: Add DAPI at a 1:20,000 dilution to the first TBS washing step.
7. Wash with deionized or distilled water.
8. Mount slides and observe under a microscope.

Note: *The SYSY standard protocol generates good staining results in the SYSY labs and may be used as a reference. However, to achieve the highest specific signal and lowest non-specific background signal, the best antibody concentration, incubation temperature and incubation time for each antibody must be individually determined. Please also refer to our general protocols.*

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