Reference Protocol for Anti-CD8a (HS-361 003) 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
- **Antigen Retrieval buffer:** citrate buffer (10 mM citrate, pH 6.0)
- **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 X100 in TBS (normal serum from the host-species of the secondary antibody is recommended for incubation)
- **Secondary antibody:** anti-rabbit 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. **Antigen Retrieval:** transfer sections into a tube filled with antigen retrieval buffer and place the tube into a water bath. Set water bath to 60°C and incubate sections overnight. Wash sections three times for 10 min in TBS (RT; orbital shaker: 70 - 80 rpm).
3. Transfer the sections to the **blocking solution** and block for 1 h at RT (orbital shaker: 70 - 80 rpm).
4. Transfer the sections to the **incubation buffer** with the primary antibody at a **1:100 dilution** and incubate overnight at 4°C (orbital shaker: 60 rpm).
5. Wash three times for 10 min in **TBS** (RT; orbital shaker: 70 - 80 rpm).
6. 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.*

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