Reference Protocol for Anti-CD8a (HS-361 017) Fluorescence Immunostaining of Fresh Frozen Cryo-Tissue Sections

Tissue

- Fresh frozen cryo-tissue sections

Materials and Reagents

- **Tris-buffered saline (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
- Water based mounting medium
- **Optional:** DAPI (4 mg/ml)
- **Fixation solutions:** 4% formaldehyde (FA) in Phosphate-buffered saline (PBS)
- hydrophobic pen

Method

1. Take fresh frozen cryo-tissue sections from -80°C freezer and air-dry sections briefly at room temperature (RT)
2. Fix sections with suitable fixative.
    - 4% FA: Fix for 15 min at RT; then start directly with the washing step.
3. Wash slides three times for 10 min in TBS at RT in staining dishes.
4. Dry sections and surround tissue with hydrophobic pen.
5. Rehydrate sections for 10 min in TBS at RT in staining dishes.
6. Add blocking buffer and block for 1 h at RT in a wet chamber.
7. Remove blocking solution and add incubation buffer with the primary antibody at a **1:200 dilution** and incubate overnight at 4°C in a wet chamber.
8. Wash slides three times for 10 min in TBS at RT in staining dishes.
9. Transfer the slides back to the wet chamber and apply the incubation buffer with the secondary antibody diluted to the manufacturer's recommended concentration. Incubate for 1 h at RT.

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.*

10. Wash slides three times for 10 min in TBS at RT in staining dishes.
11. **Optional:** Add DAPI solution for 10 min in TBS at RT.
12. Wash slides three times for 10 min in TBS at RT in staining dishes.
13. Remove the hydrophobic circle around the tissue section.
14. 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.*