IHC: Staining Protocol - Fresh Frozen

This protocol is suitable for the immunohistochemical analysis of fresh frozen cryo-tissue-sections. The tissue-sections are stained on slides. Please refer to the **remarks** section of the data sheet for suitable fixatives. For tissue preparation, please refer to our tissue preparation protocol - fresh frozen.

Materials and reagents

- **TBS**: Tris buffered saline, 50 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 antibodies 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 antibodies is recommended for blocking).
- Water based mounting medium
- **Optional:** DAPI nuclear stain
- **Fixation solutions:** 100% acetone, 100% methanol, methanol-acetone mix 1:1 or 4% formaldehyde (FA) in Phosphate buffered saline (PBS)

Procedure

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. Please refer to the **remarks section** for immunohistochemistry (IHC) on the respective data sheet.
    - **Acetone:** Fix for 10 min at -20°C; then dry tissue-section for 10-20 min before washing step (no. 3)
    - **Methanol:** Fix for 10 min at -20°C; then start directly with the washing step (no. 3.)
    - **Methanol-acetone mix 1:1**: Fix for 10 min at -20°C; then dry tissue-section for 10-20 min before washing step (no. 3)
    - **4% FA:** Fix for 15 min at RT; then start directly with the washing step (no. 3.)
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 buffer and add **incubation buffer** with the primary antibody.
8. Incubate primary antibody overnight at 4°C in a wet chamber. **Important:** Some antibodies require incubation at RT. Please refer to the corresponding antibody data sheet.
9. Wash slides three times for 10 min in **TBS** at RT in staining dishes.
10. Transfer the slides back to the wet chamber and apply the incubation buffer with the secondary antibody diluted to the manufacturer's recommended concentration.
11. Incubate for 1 h at RT. **Important Notes:**
    - This step can be omitted when fluorophore conjugated primary antibodies are used.
    - In **multiplex** staining, 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.
    - Avoid bright light when working with the secondary antibody to minimize photo bleaching of the fluorescent dye.
12. Wash slides three times for 10 min in **TBS** at RT in staining dishes.
13. **Optional:** Add DAPI solution for 10 min in TBS at RT.
14. Wash slides three times for 10 min in **TBS** at RT in staining dishes.
15. Remove the hydrophobic circle around the tissue section.
16. Mount slides and microscope.

Note: The SYSY standard protocol generates good staining results in the SYSY labs and may be used as suggestion. However, to achieve the highest specific signal and lowest non-specific background signal, the best antibody concentration, incubation temperature and incubation time must be determined individually.