IHC: Staining Protocol - Free Floating

This protocol is suitable for the immunohistochemical analysis of formaldehyde or glyoxal fixed vibratome- and cryo-tissue-sections. The tissue-sections are stained free floating. For tissue preparation please refer to our tissue preparation protocol - formaldhyde or tissue preparation protocol - glyoxal.

Important: For good detection, some proteins have special tissue preparation or antigen retrieval (AGR) requirements. Please refer to the **remarks** section for immunohistochemistry (IHC) on the respective data sheet.

Materials and reagents

- **TBS:** Tris buffered saline (50 mM Tris, 150 mM NaCl), pH 7.2
- **Blocking buffer**: 10% normal serum, 0.3% Triton X-100 in TBS (if secondary antibodies are used, 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 (if secondary antibodies are used, normal serum from the host-species of the secondary antibodies is recommended for blocking)
- Antibody and the corresponding species-specific secondary antibody
- *Optional: DAPI nuclear stain*
- **Mounting medium**

Procedure

1. Transfer the free floating sections into a staining dish containing **TBS** and wash 10 min at room temperature (RT) (orbital shaker: 70 - 80 rpm).
2. *Optional: Perform antigen retrieval (AGR).*
3. Transfer the sections to the **blocking buffer** and block for 1 h at room temperature (RT) (orbital shaker: 70-80 rpm).
4. Transfer the sections to the **incubation buffer** with the primary antibody.
5. Incubate overnight at 4°C (orbital shaker: 60 rpm). **Important:** Some antibodies require incubation at RT. Please refer to the corresponding antibody data sheet.
6. Wash three times for 10 min in **TBS** (RT) (orbital shaker: 70 - 80 rpm).
7. Transfer the sections to the **incubation buffer** with the secondary antibody diluted to the manufacturer's recommended concentration.
8. Incubate for 1 h at RT (orbital shaker: 70 - 80 rpm).
   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.*
9. Wash three times for 10 min in **TBS** (RT) (orbital shaker: 70 - 80 rpm). *Optional: Add DAPI to the first TBS washing step.*
10. Wash sections with tap water.
11. Mount slides.

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.