Protocol for Orexin A/B Antibody (Cat. No. 389 104) Immunohistochemistry Formaldehyde (IHC) Fluorescence Staining - Slide Mounted

This protocol is suitable for the immunohistochemical analysis of formaldehyde (FA) fixed tissue-sections. The tissue-sections are stained on slides. For tissue preparation, please refer to our Protocol Immunohistochemistry Formaldehyde (IHC) - Tissue Preparation.

Note on antigen retrieval (AGR): Some antigens are masked by FA crosslinking and require AGR to be accessible to the antibody. If the antibody shows no or very weak signal without AGR, we recommend AGR. In some cases, we know that the signal strength can be increased with AGR, and we point out that AGR is optional. In rare cases, we have the information that the antibody gives similar results with or without AGR, here we refer that AGR is tolerated. If you find no information about AGR in the following protocol, the antibody shows good performance without.

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)
- **Primary antibody:** Orexin A/B antibody (cat. no. 389 104)
- **Secondary antibody:** Fluorophore conjugated Guinea pig specific secondary antibody
- *Optional: DAPI nuclear stain*
- **Hydrophobic barrier pen**
- **Mounting medium**

Procedure

1. Take cryo-tissue sections from -20°C freezer.
2. Air-dry sections at room temperature (RT).
3. Surround tissue with hydrophobic pen.
4. Rehydrate sections for 10 min in **TBS** at RT in staining dishes.
5. Add **blocking buffer** and block for 1 h at RT in a wet chamber.
6. Remove the **blocking buffer** and add the **incubation buffer** with the **primary antibody** at a dilution of **1:500**.
7. Incubate with the **primary antibody** overnight at 4°C in a wet chamber.
8. Wash three times for 10 min in **TBS** at RT (orbital shaker: 70 - 80 rpm).
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.

10. Incubate for 1 h at RT.
    Notes:
    - *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.*

11. Wash slides once for 10 min in **TBS** in staining dishes at RT (orbital shaker: 70 - 80 rpm).

12. *Optional: Add DAPI solution for 10 min in TBS at RT.*

13. Wash slides twice for 10 min in **TBS** in staining dishes at RT (orbital shaker: 70 - 80 rpm).

14. Wash slides with tap water.

15. Remove the hydrophobic circle around the tissue section.

16. 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.