Protocol for ENT4 Antibody (Cat. No. 463 005) Immunohistochemistry Formaldehyde (IHC) Fluorescence Staining - Free Floating

This protocol is suitable for the immunohistochemical analysis of formaldehyde (FA) fixed vibratome- or cryo-tissue-sections. The tissue-sections are stained free floating. 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:** ENT4 antibody (cat. no. 463 005)
- **Secondary antibody:** Fluorophore conjugated Guinea pig 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. Transfer the sections to the **blocking buffer** and block for 1 h at RT (orbital shaker: 70-80 rpm).

3. Transfer the sections to the **incubation buffer** with the **primary antibody** at a dilution of **1:500 to 1:1000**.

4. Incubate **primary antibody** overnight at 4°C (orbital shaker: 60 rpm).

5. Wash three times for 10 min in **TBS** at 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.

7. Incubate for 1 h at RT (orbital shaker: 70 - 80 rpm).
   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 reagent to minimize photo bleaching of the fluorescent dye.*

8. Wash three times for 10 min in **TBS** at RT (orbital shaker: 70 - 80 rpm). *Optional: Add DAPI to the first TBS washing step.*

9. Wash sections with tap water.

10. 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.