Protocol for Collagen type IV Antibody (Cat. No. 462 017) Immunohistochemistry Fresh-Frozen (IHC-Fr) Fluorescence Staining - Slide Mounted

This protocol is suitable for the immunohistochemical analysis of fresh-frozen tissue sections. The tissue-sections are stained on slides. For tissue preparation, please refer to our Protocol for Immunohistochemistry Fresh Frozen (IHC-Fr) Tissue Preparation.

Materials and reagents

- **Fixation solution:** If more than one fixation solution is listed, use one of them. For recommendation and differences in signal strength, please refer to the remarks section for IHC-Fr
  - **Acetone** 100% pre-cooled at -20°C
  - **Methanol** 100% pre-cooled at -20°C
  - **Methanol-acetone mix 1:1** pre-cooled at -20°C
  - **Formaldehyde (FA) 4%** in Phosphate buffered saline (PBS)
- **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:** Collagen type IV antibody (cat. no. 462 017)
- **Secondary antibody:** Fluorophore conjugated rat specific secondary antibody
- *Optional: DAPI nuclear stain*
- **Hydrophobic barrier pen**
- **Water based mounting medium**

Procedure

1. Take fresh-frozen tissue sections from -80°C freezer and air-dry sections briefly at room temperature (RT).

2. Fix sections with suitable **fixative**:
   - **Acetone:** Fix for 10 min at -20°C. Dry tissue-section for 10-20 min and surround tissue with hydrophobic pen. Wash slides three times for 10 min in **TBS** in staining dishes at RT (orbital shaker: 70 - 80 rpm).
   - **Methanol:** Fix for 10 min at -20°C. Wash slides three times for 10 min in **TBS** in staining dishes at RT (orbital shaker: 70 - 80 rpm). Dry sections and surround tissue with hydrophobic pen. Rehydrate sections for 10 min in **TBS** at RT in staining dishes.
   - **Methanol-acetone mix 1:1:** Fix for 10 min at -20°C. Dry tissue-section for 10-20 min and surround tissue with hydrophobic pen. Wash slides three times for 10 min in **TBS** in staining dishes at RT (orbital shaker: 70 - 80 rpm).
   - **FA 4%:** Surround tissue with hydrophobic pen and fix for 15 min at RT. Wash slides three times for 10 min in **TBS** in staining dishes at RT (orbital shaker: 70 - 80 rpm).

3. Add **blocking buffer** and block for 1 h at RT in a wet chamber.

4. Remove the **blocking buffer** and add the **incubation buffer** with the **primary antibody** at a dilution of **1:500 to 1:1000**.

5. Incubate with the **primary antibody** overnight at 4°C in a wet chamber.

6. Wash three times for 10 min in **TBS** at RT (orbital shaker: 70 - 80 rpm).

7. Transfer the slides back to the wet chamber and apply the **incubation buffer** with the **secondary antibody** diluted to the manufacturer's recommended concentration.

8. 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.*

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

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

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

12. Wash sections with tap water.

13. Remove the hydrophobic circle around the tissue section.

14. 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.