Protocol for Claudin5 Antibody (Cat. No. 482 005) Immunohistochemistry Glyoxal (IHC-G) Tissue Preparation

Tissue preparation and fixation are important for the success of immunohistochemical experiments. First, it is crucial, that the animal is perfused with saline or phosphate buffered saline (PBS) to remove residual IgG containing blood from blood vessels. Otherwise, IgGs may be bound by cross-reactive secondary reagents and cause undesired background staining. Second, the tissue is fixed to maintain tissue integrity. In contrast to commonly used fixation reagents, e.g. formaldehyde (FA), glyoxal can be a good alternative for sensitive antigens masked by FA crosslinking. In our tissue preparation protocol for glyoxal, the animal is perfused with saline or PBS, followed by perfusion and postfixation with glyoxal. We use two different glyoxal solutions for fixation, glyoxal solution A: 3% glyoxal, 1% acetic acid, 20% ethanol in ddH_2O according to Richter et al. 2017, or glyoxal solution B: 9% glyoxal, 8% acetic acid in ddH_2O according to Konno et al. 2023. Glyoxal solution A seems to be very good for antigens located in or close to blood vessels, e.g. the blood brain barrier. Glyoxal solution B appears to be optimal for some critical antigens located in the postsynaptic density. The immersion fixed tissue can be cut at the vibratome after 48 h of fixation, or can be frozen and cut at the cryostat. The tissue is stained according to our standard protocols (Protocol for Immunohistochemistry Glyoxal (IHC-G) Fluorescence Staining - Free Floating or Slide Mounted).

Vibratome sections

Material and reagents

- Cold 0.9% saline containing 17 U/ml Heparin
- **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-G
  - **9% Glyoxal solution:** 9% glyoxal, 8% acetic acid in ddH_2O, pH 4.2-4.4, according to Konno et al. 2023
- **PBS:** Phosphate buffered saline (200 mM NaCl, 2.5 mM KCl, 8 mM Na_2HPO_4, 1.5 mM KH_2PO_4), pH 7.4
- **Cryoprotectant buffer:** 25% glycerol, 25% ethylene glycol, 50% PBS pH 7.4

Procedure

1. Transcardially perfuse with 30-50 ml cold 0.9% saline containing 17 U/ml Heparin with a rate of 5 ml/min until the tissue is cleared from blood.

2. Perfuse with suitable **fixation solution** 30-50 ml with a rate of 5 ml/min:
   - **9% Glyoxal solution**

3. After tissue dissection, postfix tissue with suitable **fixation solution**:
   - **9% Glyoxal solution:** for 3 days at 4°C (alternatively 2-14 days)

4. After postfixation, cut tissue with a vibratome (50 µm) in ice-cold **PBS** and store afterwards at -20°C in **cryoprotectant buffer** until staining procedure (Protocol for Immunohistochemistry Glyoxal (IHC-G) Fluorescence Staining - Free Floating).

Cryostat sections

Materials and reagents

- Cold 0.9% saline containing 17 U/ml Heparin
- **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-G
  - **9% Glyoxal solution:** 9% glyoxal, 8% acetic acid in ddH_2O, pH 4.2-4.4, according to Konno et al. 2023
- **PBS:** Phosphate buffered saline (200 mM NaCl, 2.5 mM KCl, 8 mM Na_2HPO_4, 1.5 mM KH_2PO_4), pH 7.4
- **Sucrose solution:** 30% sucrose in PBS
- **Cryoprotectant buffer:** 25% glycerol, 25% ethylene glycol, 50% PBS pH 7.4
- Tissue-Tek®
- Slides SuperFrost® plus

Procedure

1. Transcardially perfuse with 30-50 ml cold 0.9% saline containing 17 U/ml Heparin with a rate of 5 ml/min until the tissue is cleared from blood.

2. Perfuse with suitable **fixation solution** 30-50 ml with a rate of 5 ml/min:
   - **9% Glyoxal solution**

3. After tissue dissection, postfix tissue with suitable **fixative**:
   - **9% Glyoxal solution:** for 3 days at 4°C (alternatively 2-14 days)

4. Incubate tissue in **sucrose solution** for 1-3 days at 4°C.

5. Freeze tissue on dry ice and store at -80°C until cutting.

6. Cut tissue with a cryostat:
   - Sections on slides: Cut tissue 10-20 µm and mount on slides. Dry sections for 30 min at 57°C. Store slides at -20°C until staining procedure (Protocol for Immunohistochemistry Glyoxal (IHC-G) Fluorescence Staining - Slide Mounted).
   - Sections free floating: Cut tissue 25-50 µm with a cryostat and store in **cryoprotectant buffer** at -20°C until staining procedure (Protocol for Immunohistochemistry Glyoxal (IHC-G) Fluorescence Staining - Free Floating).

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.