# Protocol for GABA-A receptor gamma2 Antibody (Cat. No. 224 004)
# Immunohistochemistry Fresh-Frozen (IHC-Fr)
# 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), methanol or acetone can be good alternatives for sensitive antigens masked by FA crosslinking. In this case, the animal is perfused with saline or PBS and the tissue is immediately snap frozen after dissection (called fresh-frozen tissue). The fresh-frozen tissue is cut with the cryostat, mounted on slides and can be postfixed with the suitable fixative – methanol, acetone or FA (please refer to the remarks sections for recommended fixatives and find the fixation protocol in the (Protocol for Immunohistochemistry Fresh Frozen (IHC-Fr) Fluorescence Staining - Slide Mounted).

## Material and reagents

- Cold 0.9% saline containing 17 U/ml Heparin
- Isopentane
- Dry ice
- liquid nitrogen
- 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. Dissected and with Tissue-Tek® immersed tissues are placed on small plastic weighing bowls and immediately snap frozen on liquid nitrogen pre-cooled isopentane.

3. Store snap frozen tissue at -80°C until cutting.

4. Cut tissue 12 µm with a cryostat and mount on slides.

5. Dry sections for 3-10 min at room temperature (RT) and store at -80°C until staining procedure (Protocol for Immunohistochemistry Fresh Frozen (IHC-Fr) Fluorescence Staining - Slide Mounted).

*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.*