Anti-GABA\textsubscript{A} Receptor, $\beta_3$-Subunit Antibody Immunohistofluorescence Protocol

**Catalog #:** 863A-GB3C  
**Species:** Rabbit  
**Tissue:** Mouse Retina

**Fixation:** 4% Paraformaldehyde in 1X PBS for 15 minutes
**Antibody incubation:** Primary Antibody- 4C, overnight  
**Secondary Antibody-** RT, 1 hour

**Materials Required**
- **Fixative:** 4% Paraformaldehyde in freshly prepared 1X PBS
- **OCT compound:** Fisher Healthcare  
  **Cat #:** 23-730-571
- **1X PBS:** 137 mM NaCl, 28 mM Na\textsubscript{2}HPO\textsubscript{4}, 5.4 mM KCl, 2.9 mM KH\textsubscript{2}PO\textsubscript{4}, pH 7.6
- **10%/ 20%/ 30% sucrose buffer:** 10/20/30g of sucrose in 100mls of 1xPBS
- **Blocking buffer:** 1X PBS with 10% goat serum, 1% BSA, 0.5% Triton X-100 also use for secondary incubation buffer
- **Primary Incubation buffer:** 1X PBS with 3% goat serum, 1% BSA, 0.05% sodium azide, 0.5% Triton X-100
- **Secondary Incubation buffer:** 1X PBS with 3% goat serum, 1% BSA, 0.05% sodium azide, 0.5% Triton X-100
- **Secondary Antibody:** example used is Goat-Anti-Rabbit Alexa 488 from ThermoFisher
- **Mounting media:** ProLong Gold Antifade Mountant Medium  
  **Cat #:** H-1200

**Before you begin**
This protocol can be used for tissues fixed with or without perfusion. If tissues are harvested without perfusion, slice tissues into 0.5cm sections and place in 4% paraformaldehyde for 45 minutes at RT. Make sure the slices have sufficient volume of fixative for proper fixation. If tissues are harvested after perfusion, harvest and place in fixative for 15-30 minutes. For optimal antibody epitope binding, tissues should not be stored in fixative. It is best to store fixed tissue in cryoprotectant solution of 30% sucrose buffer.

**Protocol**
1. Place fixed tissue specimen into 30% sucrose in 1X PBS for 2-3 days at 4C.  
   **Tech Tip:**  
   a. To prevent ice crystals from forming on tissue and destroying antibody epitope binding sites, do not remove the tissue until it has sunk to the bottom of the beaker to ensure complete sucrose infiltration.
   b. To speed up the cryoprotection process, place fixed tissue in graded sucrose buffers. Starting with 10% sucrose, once tissue has sunk to the bottom transfer to 20% sucrose buffer. Do the same for 20%/ 30% sucrose. Once the tissue has sunk in the 30% sucrose it is ready for processing.
2. Transfer tissue into OCT compound and freeze at -80C overnight.
3. Mount tissue onto cryostat and cut tissue into 10-15 micron thick sections at -25C. Mount tissue sections onto gelatin coated slides.
   
   Tech Tip:
   a. Slides can be stored at -70C for long term storage.

4. Thaw sections for 10 minutes at RT.

5. Wash the sections 3 times with 1X PBS for 10 minutes.

6. Block slides with blocking buffer for 1 hour at RT.

7. Wash the slides 3 times with 1X PBS for 10 minutes.

8. Dilute Anti-GABA, Receptor, β3-Subunit Antibody (Cat. # 863A-GB3C) to 1:300 in primary incubation buffer. Incubate sections overnight at 4C.

9. Wash the slides 3 times with 1X PBS for 10 minutes.

10. Dilute secondary antibody in secondary incubation buffer per manufacturer’s recommendation. Incubate sections for 1 hour at room temperature.
   
   Tech Tip:
   a. Alexa Fluor 488 dye diluted 1:1000 was used to produce the image below.

11. Remove secondary antibody and wash with 1x PBS 3 times, in 10 minute intervals.

12. Apply mounting medium onto slide and gently place glass cover slip before viewing under the microscope.

   Tech Tip:
   a. Any mounting media can be used, for this protocol ProLong Anti-Fade medium was used. Cat #: H-1200.

**Immunofluorescence of mouse retina showing staining of GABA, R, β-subunit (catalog #: 863A-GB3C, green, 1:300) and calbindin (red). The blue is DAPI staining nuclear DNA. Photo courtesy of Dr. Arlene Hirano, UCLA.**