Anti-VGAT
Immunohistofluorescence Protocol

Catalog #: 2100-VGAT
Species: rabbit
Tissue: Rat brain

Fixation: 4% Paraformaldehyde, 0.05% picric acid in 1X PBS for 2 hours
Antibody incubation: Primary Antibody- 4C, overnight
Secondary Antibody- RT, 2 hours or 4C, overnight
Antigen Retrieval: None

Materials Required

- ✓ **Fixative:** 4% Paraformaldehyde, 0.05% picric acid in freshly prepared 1X PBS
- ✓ **isopentane:** chilled to -160C
- ✓ **acetone:** chilled to -20C
- ✓ **1X PBS:** 137 mM NaCl, 28 mM Na_2HPO_4, 5.4 mM KCl, 2.9 mM KH_2PO_4 , pH 7.6
- ✓ **30% sucrose buffer:** 30g of sucrose in 100mls of 1xPBS
- ✓ **Blocking buffer and Incubation Buffer:** 1X PBS with 2% fetal bovine serum and 1% Triton X-100
- ✓ **Secondary Antibody:** example used is Goat-Anti-Rabbit Alexa 488 from ThermoFisher

Before you begin

The tissue used in this protocol were perfused intracardially. Alternatively, tissues can be fixed without perfusion. Immediately after sacrifice slice tissues into 0.5cm sections and place in 4% paraformaldehyde for 2 hours at RT. Submerge sections into a sufficient volume of fixative, for proper fixation a recommended minimum volume of 20x each in separate containers. For optimal antibody epitope binding, tissues should not stay longer than 24 hours in fixative.

Protocol

1. Place fixed tissue section into 30% sucrose in 1X PBS overnight 48 hours 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.
2. Transfer tissue into isopentane that is at -160C. This can be done by cooling the isopentane in a plastic beaker placed in liquid nitrogen. Once tissue is frozen remove and immediately section with cryostat or store at -70C.
3. Mount tissue onto Leica cryostat and cut serial coronal sections into 60-80 mu thick sections at -25C. Place sections into PBS in a container to float freely.
4. Rinse tissue sections with PBS 3 times, in 5 minute intervals.
5. Block tissue sections with blocking buffer for 1 hour at RT.
6. Rinse tissue sections with PBS 3 times, in 5 minute intervals.

7. Dilute Anti-VGAT (Cat. # 2100-VGAT) to 1:1000 in incubation buffer. Incubate sections for 2 hours at room temperature or overnight at 4C.
8. Rinse tissue sections with PBS 3 times, in 5 minute intervals.
9. Dilute secondary antibody in incubation buffer per manufacturer's recommendation. Incubate tissue sections for 2 hours at room temperature or overnight at 4C.

   Tech Tip:

   a. Alexa Fluor 488 dye diluted 1:1000 and 0.5% Triton X-100 was changed in the incubation buffer.
10. Remove secondary antibody and wash the tissue section with PBS 3 times, in 5 minute intervals.
11. Place tissue sections onto slide and apply mounting medium. Gently place glass cover slip before viewing under the microscope.

    Tech Tip:

    a. Any mounting media can be used, for this protocol fluorescent mounting media with and without DAPI counterstaining was used.

Reference:

Noseda, R., Kainz, V., Borsook, D., & Burstein, R. (2014). Neurochemical pathways that converge on thalamic trigeminovascular neurons: potential substrate for modulation of migraine by sleep, food intake, stress and anxiety. *PLoS One*. Aug 4;9(8):e103929.