Fluorescence immunohistochemistry for free-floating sections (100 μm)

Useful notes:

- Perfused mice with 4%PFA freshly prepared or thawed (this is important to reduce the non-specific biding of anti-mouse secondary antibody and autofluorescence from blood) and post-fix with 4%PFA for overnight at 4°C.
- After post-fixing overnight, rinse the brain with 1X PBS (good amount) and keep in 1X PBS with 0.1% sodium azide in 4°C until sectioned (brains should be sectioned as soon as possible so antigens in brain sections can be preserved).
- After sectioning, store brain sections in antigen preservation solution in -20°C. Make sure to use enough solution to immerse all sections and all sections get equilibrated with preservation solution before putting into freezer.
- When selecting brain sections, keep them in antigen preservation solution.
- To start the staining procedure, make sure sufficient rinse/wash the sections to get rid of preservation solution. Rinsing with large volume of PBS with a few rounds will be helpful. In general, washing is an important step to obtain good staining results.
- All wash and incubations are carried out on a shaker.
- Use transfer pippet to remove solutions from the top without touching sections. Never leave sections dry out.
- When sections in the well plate, make sure all sections moving independently without sticking to each other.

Procedures:

Day1-

- Gently remove desired sections from antigen preservation solution with great care to avoid damage on sections.
- Transfer sections to 12 or 24 well plate (or appropriate container) with PBS in it. It should be sufficient to cover all sections. Rinse them out from preservation solution. Take out as much PBS possible but not touching sections with transfer pippet.
- Add in PBS and wash for 10 minutes on shaker with gentle shaking (can be twice or three times 5 min each to get rid of any trace of preservation solution; remember, rinsing is good).
- Incubate in **the permeabilization buffer (2% Triton X-100 in PBS)** for 30 minutes.
- Incubate sections in **the blocking buffer (PBS-T with 3% normal goat serum or normal donkey serum, depending on what secondary antibody will be used**), RT for 1 h.
- Incubate sections in **primary antibodies prepared in blocking buffer** at 4°C for 48hours on shaking platform.

NOTE

- For 12-well plate, primary antibody volume should be ~450ul-500ul, max. four sections per well.
- If choosing 24-well plate, primary antibody volume should be ~ 250ul-300ul, and one section per well.
- Make sure to label the plate, accordingly, including mouse ID, date, and project name.

Example VGluT1+PSD95+VGluT2 triple staining:

1° Ab Rabbit anti-VGluT1 (SYSY, 135-302) 1:2000
2° Ab Goat anti-rabbit Alexa 488, Life Technologies, A11034

1° Ab Mouse (IgG1) anti-PSD95 (SYSY, 124-011) 1:1000
2° Ab Goat anti-mouse -IgG1-Alexa 555, Life Technologies, A21127

1° Ab Guinea Pig anti-VGluT2 (SYSY, 135-404) 1:2000
2° Ab Goat anti- GP Alexa 647, Life Technologies, A21450

Day3-

- Wash 3 times with PBS-T for >10 mins each.
- Incubate sections with the mixture of secondary antibodies +DAPI prepared in blocking buffer for 24 hours at 4°C.
- Wrap the plate with aluminum foil from now till mounting. (Secondary antibodies are light sensitive. Protect the sections all time when adding the secondary antibodies!)

Appropriate secondary antibody with 1:2000
DAPI 50 ng/ml, 1:5,000 dilution of stock

Day4-

- Wash > 3 times with PBS-T for >10 mins each
- Mount sections with mounting medium or preserve in 1X PBS.

Notes:

- When mounting the slides, make sure the glass surface is wet and is at a shallow angle. Let it be seated in a flat surface protected from the light for 2 overnights.
- Label the slides accordingly, with date, mouse ID, brain region, ab used (consider using the slide label generator in Katona lab purchased with C3A to generate bar codes)
- Make sure to have all the information in the lab notebook and computer spreadsheet.