

Xu lab protocol and instructions

Processing of recorded slices for immunochemical identification // Biocytin staining

Fix brain slices in 4% PFA overnight, then transfer them into 30% sucrose in PBS (Slices can stay in the sucrose solution for a few days. Generally the sooner the processing is done, the better. Can add cryoprotective solution to store them in a -20°C freezer)

Before immunostaining of the recorded slices,

1. Get a new and clean 24-well tray, and transfer slices to the tray with a paint brush. Label the tray and individual wells. Please write legibly.
2. Rinse slices with PBS 3-4 times (3')
3. Incubate slices in blocker for **1 hour** [blocker solution for 50 ml: Normal donkey serum 5 ml, BSA, 1 g, Triton X 100, 125 ul, 1xPBS, 45 ml] on a shaker. (blocker: 0.4 ml per well)
4. Immunochemical staining: staining biocytin inside the recorded cell with 1:1000 or 1:500 streptavidin-cy3 (Cy3-conjugated Streptavidin Code Number: **016-160-084**; Jackson ImmunoResearch Laboratories, Inc) // Incubate slices with streptavidin-cy3 in blocker solution for at least 5 hours at room temperature on a shaker. (streptavidin-cy3 blocker solution 0.4 ml per well). Or could leave the slices overnight at -4 fridge after tested trails for your samples
5. Rinse slices with PBS 3-4 times (5') to stop the cy3 staining
6. DAPI staining for 10 minutes (to make DAPI solution, add 100 ul DAPI stock solution in 10 ml PBS). 0.4 ml per well // Rinse the slices off with PBS 3-4 times
7. Wet-mount slices (on non-subbed slides) and cover-slip with Vectashield.
8. Fluorescent scope examination, and take low power pics (x4, x10, x20)
9. Confocal scanning (x10, x20, x40)

[Optional procedures:

Remove slices, rinse, and transfer them into 30% sucrose PBS overnight

Resection 400 um slices into 80 um sections

Check some re-sectioned slices to see if cy3 label still exists

Take a few superficial sections for CO staining (for identification laser alignment markers). Use the slice with a cell body to do further immuno staining.]