DRBIO
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Application Note #4

Labeling Neuronal Populations with AM-Ester Fluorescent Dyes.

(Dr. Alex Kwan, Applied and Engineering Physics, Cornell Unviersity)

This protocol is modified from the procedures described in the literature (Stosiek et al., 2003; Garaschuk et al., 2006). These steps have been used in DRBIO to label neuronal populations with AM-ester fluorescent dyes in hippocampal slices and in neonatal spinal cords. Successful dye loading will result in a uniformly-labeled area, ~400μm in diameter (Stosiek et al., 2003), that contains many cells and few bright specks (Fig. 1). Dye can be loaded through pressure injection, as described here, or by simple topical application, as described by MacLean and Yuste (MacLean and Yuste, 2005).

1. Pull patch pipette, outer diameter 1.5 mm, borosilicate glass, filament, 1-4 MΩ.

2. From 5 mL sealed glass vials of DMSO (e.g. from Sigma), aliquot into 0.5 mL tubes and store in desiccator. Fresh DMSO, at most week-old, is essential.

3. Put 50 mg pluronic into 0.25 mL DMSO to make 20% solution, stir until dissolved on hot plate at 60˚C for about 10 min until solution becomes clear.

4. Add the pluronic/DMSO solution (~4.0 μL, depending on molecular weight of the dye) to 5 mg AM-ester fluorescent dyes (e.g. “Special-packaged” Oregon Green BAPTA-1 AM or Fluo-3 AM from Invitrogen) to make 10 mM.

5. Vortex for 15 s so the pluronic/DMSO would catch all the dyes that were stuck to the sides of the vial.

6. Centrifuge at moderate speed, once centrifuge is up to full speed, stop it so the dye solution sits at bottom of the vial.

7. Fill sonicator with room temperature water.

8. Sonicate for 5 min.

9. Add pipette solution, consisting of 150 mM NaCl, 2.5 mM KCl, 10 mM HEPES, to dilute 1:10 (~36 μL).

10. Vortex for 15 s and looking at vial to verify a clear, well-mixed dye solution.

11. Sonicate for 10 to 20 min.

12. Pipette dye solution onto a 0.22 µm microcentrifuge filter (e.g. from Sigma-Aldrich). The solution should have no floating particulates. Spin for 10 s at high speed such that solution filters through the membrane.

13. Use thin, plastic tips (e.g. µLoader, Eppendorf) to fill pipette.

14. Insert pipette into tissue with a holding pressure of 4 psi and eject dye for more than 5 min at 10 psi. Lower pressure will reduce tissue damage but will require longer time to eject the same amount of dye.

15. Incubate the tissue and wait for the dye to be either internalized or washed away. For neonatal mouse spinal cords at room temperature, optimal loading can be seen in 1 hr.

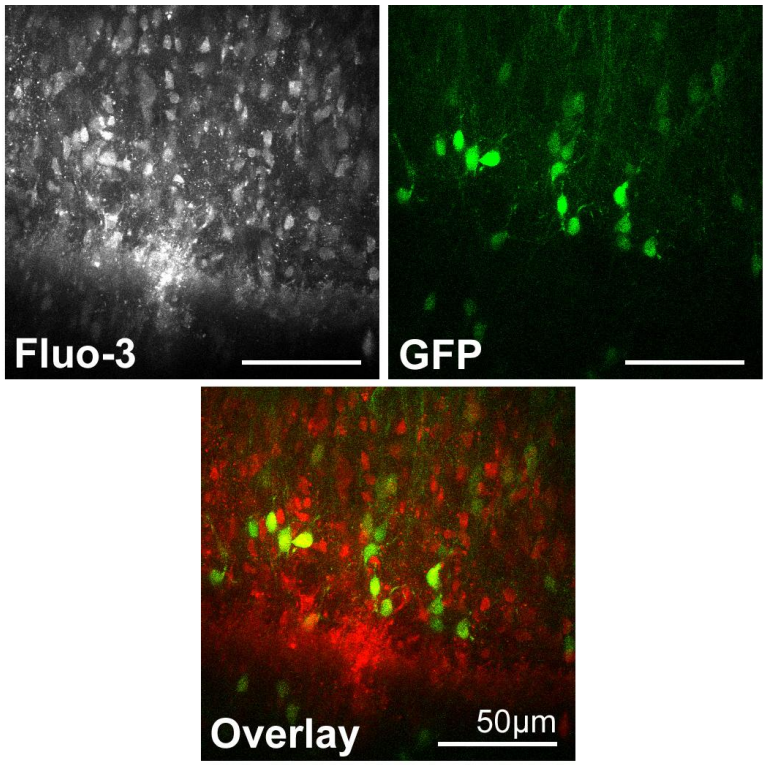

Figure 1: Ventral spinal cord labeled by bolus injection of AM-ester fluorescent calcium indicator dyes. In this transgenic Hb9-GFP mouse line, a set of excitatory interneurons is expressing GFP, which can be spectrally separated from the injected Fluo-3-AM. (unpublished data by A.C.K, S.B.D., W.W.W., R. H.-W.)

References cited

Garaschuk, O., Milos, R.-I., Konnerth, A. (2006). “Targeted bulk-loading of fluorescent indicators for two-photon brain imaging in vivo.” Nat Protocol **1**, 380–386.

MacLean, J. N., Yuste, R. (2005). “A practical guide: imaging action potentials with calcium indicators.” In Imaging in Neuroscience and Development, A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Stosiek, C., Garaschuk, O., Holthoff, K., Konnerth, A. (2003). “In vivo two-photon calcium imaging of neuronal networks.” Proc Natl Acad Sci USA **100**, 7319–7324.