Polarity IHC of Drosophila CNS

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Abstract

The use of genetically encoded 'self-labeling tags' with chemical fluorophore ligands enables rapid labeling of specific cells in neural tissue. To improve the chemical tagging of neurons, we synthesized and evaluated new fluorophore ligands based on Cy, Janelia Fluor, Alexa Fluor, and ATTO dyes and tested these with recently improved *Drosophila melanogaster* transgenes. We found that tissue clearing and mounting in DPX substantially improves signal quality when combined with specific non-cyanine fluorophores. We compared and combined this labeling technique with standard immunohistochemistry in the Drosophila brain.

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Guidelines

- All tissues and solutions are at room temperature (RT), unless noted. Always protect tissue from light exposure.
- For details on dissection and fixation see FlyLight Protocol - Adult Dissection and 2% Fixation.
- For mounting and embedding instructions refer to FlyLight Protocol – DPX Mounting.
- Protocols at https://www.janelia.org/project-team/flylight/protocols
- Reporter Genotype: w; brp-SNAP / CyO; pJFRC225-5xUAS-IVS-myr::smGFP-FLAG in VK00005, pJFRC51-3xUAS-Syt::smGFP-HA in su(Hw)attP1
- For details on polarity constructs, please refer to Aso et al. 2014. http://elifesciences.org/content/3/e04577

Before start

Reagents and Supplies

- Cy2 Goat α-Mouse Jackson Immuno Research. # 115-225-166
- Cy3 Goat α-Rabbit Jackson Immuno Research. # 111-165-144
- ATTO 647N Goat α-Rat IgG (H&L) Antibody. Rockland. # 612-156-120
- DPX Mountant for Microscopy. Electron Microscopy Sciences. # 13512, 500 mL
- Ethanol, ACS reagent, >99.5% (200 proof). Sigma Aldrich. # 459844-1L
- GS – Goat Serum. Life Technologies. # 16210-064, 100 mL
- Kodak Photo-Flo 200 Solution. Electron Microscopy Sciences. # 74257
- nc82 – Mouse α- Developmental Studies Hybridoma Bank. # nc82-s
- PBS - Phosphate Buffered Saline, 1X**.** # 21-040
- PFA – Paraformaldehyde. 20% PFA. Electron Microscopy Sciences. # 15713-S
- Poly-L-Lysine. Sigma Aldrich. # P1524-25MG
- Protein LoBind Microcentrifuge Tubes. Eppendorf. # 022431102

- Rat α-FLAG Tag (DYKDDDDK Epitope Tag). Novus Biologicals. # NBP1-06712
- Rabbit α-HA Tag. Cell Signal Technologies. # 3724S
- S2 – Schneider's Insect Medium. Sigma Aldrich. # S01416
- Triton X-100. Sigma Aldrich. # X100
- Xylenes. Fisher Scientific. # X5-500

Protocol

Step 1.
Dissect. Dissect adult brains or CNS in cold Schneider's Insect Medium (S2).

Step 2.
Fix. Transfer tissue to 2 mL Protein LoBind tubes filled with 2% paraformaldehyde (PFA) in S2 at RT. Fix for 55 minutes at RT while nutating.

Step 3.
Post-fix wash. Remove the fix and add 1.75 mL phosphate buffered saline with 0.5% Triton X-100 (PBT) and wash for a total of 4 X 10-minutes washes while nutating. If needed, store tissue in 0.5% PBT at 4°C while nutating or lay tube flat and rotate.

Step 4.
Block Goat Serum (GS). Remove PBT and add 200 µL 5% GS in PBT per tube. Incubate for 1.5 hours at RT on a rotator with tubes upright.

Step 5.
Reference primary antibodies. Remove block and add primary antibody diluted in 5% GS in PBT for a volume of 200 µL per tube. Incubate for 4 hours at RT on a rotator with tubes upright. Then continue incubation at 4°C on a rotator with tubes upright for 2 overnights.

Mouse nc82 (1:30 or 33.3 µL/mL)

Step 6.
Post-reference primary washes. Remove the primary antibody and do a brief rinse with 1.75 mL 0.5% PBT. Allow the tissue to settle to the bottom and then remove the rinse solution and add 1.75 mL 0.5% PBT. Wash for a total of 3 X 30-minute washes while nutating (or 4 X 15 minutes).

Step 7.
Reference secondary antibody. Remove PBT and add the reference primary antibody diluted in 5% GS in PBT for a volume of 200 µL per tube. Incubate for 4 hours at RT on a rotator with tubes upright. Continue incubation at 4°C on a rotator with tubes upright for 2-3 overnights.

Cy2 Goat α-Mouse (1:600 or 1.67 µL/mL)

Step 8.

Second Fixation. Transfer tissue to 2 mL Protein LoBind tubes filled with 2% paraformaldehyde (PFA) in PBS at RT. Fix for 55 minutes at RT while nutating.

Step 9.
Post-fix wash. Remove the fix and add 1.75 mL phosphate buffered saline with 0.5% Triton X-100 (PBT) and wash for a total of 4 X 10-minutes washes while nutating. If needed, store tissue in 0.5% PBT at 4°C while nutating or lay tube flat and rotate.

Step 10.
Block #2 GS. Remove PBT and add 200 µL of 5% GS in PBT per tube. Incubate for 1.5 hours at RT on a rotator with tubes upright.

Step 11.
Neuron primary antibodies. Remove block and add primary antibodies diluted in 5% GS in PBT for a volume of 200 µL per tube. Incubate for 4 hours at RT on a rotator with tubes upright. Then continue incubation at 4°C on a rotator with tubes upright for 2 overnights.

Rat α-FLAG Tag (1:100 or 10 µL/mL)
Rabbit α-HA Tag (1:600 or 1.67 µL/mL)

Step 12.
Post-neuron primary washes. Remove the neuron primary antibodies and do a brief rinse with 1.75 mL 0.5% PBT. Allow the tissue to settle to the bottom and then remove the rinse solution and add 1.75 mL 0.5% PBT. Wash for a total of 3 X 30-minute washes while nutating (or 4 X 15 minutes).

Step 13.
Neuron secondary antibodies. Remove the PBT and add the secondary antibodies diluted in 5% GS in PBT for a volume of 200 µL per tube. Incubate for 4 hours at RT on a rotator with tubes upright. Then continue incubation at 4°C on a rotator with tubes upright for 2-3 overnights.

ATTO647N Goat α-Rat (1:150 or 6.6 µL/mL)
Cy3 Goat α-Rabbit (1:1000 or 1 µL/mL)

Step 14.
Post-secondary washes. Remove the secondary antibodies and do a brief rinse with 1.75 mL 0.5% PBT. Allow the tissue to settle to the bottom and then remove the rinse solution and add 1.75 mL 0.5% PBT. Wash for a total of 3 X 30-minute washes while nutating (or 4 x 15 minutes). If needed, store tissue in 0.5% PBT at 4°C while nutating or lay tube flat and rotate.

Step 15.
Pre-embedding fixation. Remove PBT and add 1.75 mL 4% PFA in PBS at RT. Fix for 4 hours at RT while nutating.

Step 16.

Post-4% PFA washes. Remove the 4% PFA and do a brief rinse with 1.75 mL 0.5% PBT. Allow the tissue to settle to the bottom and then remove the rinse solution and add 1.75 mL 0.5% PBT. Wash for a total of 4 X 15-minute washes while nutating. If needed, store tissue in 0.5% PBT at 4°C while nutating or lay tube flat and rotate.

Step 17.
Mount. Mount the tissue on a poly-L-lysine (PLL) coated cover glass.• For making PLL see FlyLight Recipe – Poly-L-Lysine.

Step 18.
Dehydrate. Move the cover glass through a series of 7 cover glass staining jars filled with increasing concentrations of ethanol (30%, 50%, 75%, 95%, 100%, 100%, 100%). Soak the cover glass for 10 minutes in each jar.

Step 19.
Xylene clearing. (In the hood). Move the cover glass through a series of 3 jars filled with xylene. Soak the cover glass for 5 minutes in each jar.

Step 20.
DPX embedding. Add 7 drops of dibutyl phthalate in xylene (DPX) on top of the tissue mounted on the cover glass. Place the cover glass (DPX down) on a prepared slide with spacers. Use the edge of a glass slide to gently press down on the center of the cover glass to seat the cover glass onto the slide. Let the slide dry in the hood for 2 days before viewing.