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Immunofluorescent staining of dopaminergic neurons in brains of D. melanogaster

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We use this protocol and it's working

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Abstract

This protocol is used to visualize for dopaminergic neurons in Drosophila brains, but can be adapted for other targets as well.

Materials

Rabbit polyclonal anti-TH, Millipore, Cat# AB152, RRID: AB_390204
Mouse monoclonal anti-DLG, DSHB, DSHB Cat# 4F3 anti-discs large; RRID: AB_528203
Goat anti-Rabbit IgG Alexa Fluor™ 488, Life Technologies, Cat# A-11034 RRID: AB_2576217
Goat anti-Mouse IgG Alexa Fluor™ 555, Life Technologies, Cat# A-21424 RRID: AB_141780

RapiClear 1.47, Sunjin Lab, Cat# RC147001
Triton X-100 Solution, Sigma Aldrich, Cat# 93443-100ML
Formaldehyde solution (37 wt. % in H2O) F1635-500ml, Sigma-Aldrich

Dissection of fly brains, fixation, block and primary antibody incubation

1h 30m

1 Prepare fresh 3.7% paraformaldehyde in 1x PBS, 0.2% Triton X-100 (PBX), 500 µL per genotype and store On ice.

2 Dissect fly brains in ice-cold PBS under stereomicroscope, collect with a PBX coated pipette and transfer in the 3.7% paraformaldehyde solution.

2.1 Store the dissected fly brains in paraformaldehyde solution on ice, but do not keep fly brains longer than 01:00:00 On ice 1h

3 Incubate the dissected fly brains in paraformaldehyde solution on rotation wheel for 00:30:00 at Room temperature 30m

4 Take the paraformaldehyde solution off and wash a first time with PBX 0.2% (500 µL) and take it off right away

5 Wash 3 times with PBX 0.2% (500 µL) for 15 min on a rotator at Room temperature

6 Block with 10% NGS in PBX (500 µL) for 01:00:00 on rotator at Room temperature 1h

7 Add primary antibody (in 500 µL of 10% NGS in PBX) put it on rotation wheel at 4 °C 48:00:00 2d

7.1 Use rabbit a-TH, Sigma, 1:200, and mouse a-DLG, DSHB, 1:100

secondary antibody incubation

2d

8 Take the primary antibody solution off and wash a first time with PBX 0.2% (500 µL) and take it off right away

9 Wash 3 times with PBX 0.2% (500 µL) for 15 min on a rotator at Room temperature

10 Add secondary antibody (in 500 µL of 10% NGS in PBX), cover with tinfoil and put it on rotation wheel at 4 °C Overnight 2d

10.1 Use Goat anti-Rabbit IgG Alexa Fluor™ 488, Life Technologies, and Goat anti-Mouse IgG Alexa Fluor™ 555, Life Technologies, at 1:500 dilution.

Mounting 15m

11 Take the secondary antibody solution off and wash a first time with PBX 0.2% (500 µL) and take it off right away

12 Wash 3 times with PBX 0.2% (500 µL) for 15 min on a rotator at Room temperature

13 prepare slides:

13.1 clean slides with EtOH

13.2 place 2 book binder rings on top of each other

13.3 fill book binder rings with 0.075% PLL solution, leave minimum 00:15:00 , take the solution off and leave a thin layer to dry 15m

14 Mount fly brains by pipetting the brains next to the book binders, fill the book binder chamber with PBX and transfer the brains individually with forceps to the book binder chamber, orient the brains correctly with anterior facing up (antenna lobe up) and softly push down until they stick

15 remove PBX

16 add 8 µl of mounting medium (RapiClear 1.47) by gently pipetting on top of brains

17 add glass coverslip, seal with nail polish and let cure overnight at 4 °C in dark