CelExplorer

FocusClear™

Foreign Patented

Protocol for *Drosophila* Brain Immunostaining

Preparing any type of chemical please read the MSDS book to determine what types of safety clothing and equipment you should wear

MATERIALS

•1X PBS (100 mM Na_2HPO_4/NaH_2PO_4, pH 7.2)

•Triton™ X-100 (Sigma-Aldrich, cat. no. T8787)

•Sodium azide (Sigma-Aldrich, cat. no. S2002)

•Paraformaldehyde (Electron Microscopy Sciences, cat. no. 15713-S)

•Normal goat serum (Lampire Biological Laboratories, cat. no. S2-0609)

•Primary antibody: for example, the 4F3 mouse anti-discs large (DLG) antibody (Developmental Studies Hybridoma Bank. Univ. of Iowa, USA; 1:50) is used to label general neuropils in *Drosophila* brain or rabbit anti-HA antibody (Abcam; 1:2000).

•Secondary antibodies and streptavidin conjugated florescence dye: these may include Alexa-546 anti-mouse and biotinylated goat anti-rabbit (Molecular Probes; 1:250) and Alexa Fluor 635 streptavidin (Molecular Probes; 1:500).

•*FocusClear*™ (CelExplorer, catalog no. FC-101), an aqueous sugar-based solution rendering biological tissue transparent.

•*MountClear*™ (CelExplorer, catalog no. MC-301), a mounting solution compatible with *FocusClear*™.

EQUIMENTS

•Standard fly culturing equipment and microscope

•25 °C incubator to maintain fly strains

•Dissecting stereomicroscope (Carl Zeiss, Stemi 2000)

•Vacuum oven and pump (Risen Inc. RUD-30L)

•24-well immunostaining plate

•Microwave oven (2,450 MHz, 1100 Watts). The microwave energy was measured by heating one liter of water, to be 34.98±1.60 kcal, at room temperature.

•Orbital shaker (Bio-East Technology Co., Ltd, Taiwan, cat. no. OSR201)

•1.5-ml microcentrifuge tubes

•Two pairs of sharp forceps (Dumont, no. 55)

•Loop (~0.5 mm diameter)

•Dissection dishes

•Kimwipes

•Coverslips (PaulMarienfeld GmbH & Co. KG, catalog no. 01 010 50)

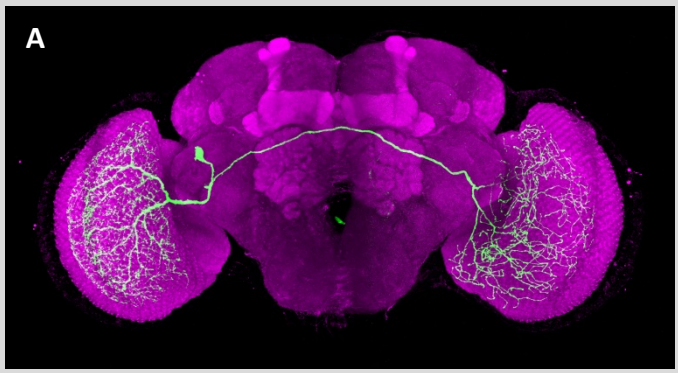
•Slides (Paul Marienfeld GmbH & Co. KG, catalog no. 10 012 02)

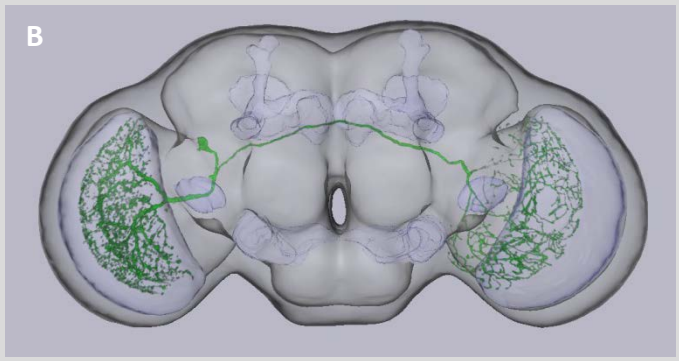
•Neo-Mount™ medium (Merck Co., Ltd.) or clear nail polish

•Reinforcing rings (Wen Lung Printing Inc. catalog no. WL-8210)

•Confocal microscope equipped with an argon-krypton laser (458, 488, or 514 nm) and two HeNe lasers (543 and 633 nm).

•40X water-immersion objective lens (N.A. value ≧ 1.2) or63X water-immersion objective lens (N.A. value ≧ 1.4).





Imaging fly brain

(A) Expression pattern of a *Drosophila* optical lobe projection neuron.Neuron arborization is labeled by mCD8::GFP (green), brain structures are counterstained by anti-DLG immunostaining (magenta).

(B) 3D visualization of the optical lobe projection neuron showed in figure A.

STOCKS

•20% PBT	Add 20 ml Triton-X 100 to 80 ml PBS and store at 4°C.
•1% sodium azide	Add 0.5 g sodium azide to 50 ml PBT. Store at 20°C. **!CAUTION** Toxic.
•Fixation solution	Add 10 ml 16% w/v paraformaldehyde to 30 ml 0.25% (vol/vol) PBT in a 50 ml tube. Must be prepared fresh and placed at 4 °C. **!CAUTION** Toxic.
•Washing Buffer	Add 30 g NaCl and 5 ml 20% PBT to 995 ml PB. Store this nonhazardous buffer at 4 °C.
•Blocking buffer	10% (vol/vol) NGS containing 0.5 ml 20% PBT, 0.5 ml NGS and 0.1 ml 1% sodium azide in 3.9 ml PBT. Store this solution for only a short period (overnight at the very most) at 4°C.
•Dilution Buffer	Add 0.0625 ml 20% PBT, 0.05 ml NGS and 0.1 ml 1% sodium azide to 4.7875 ml PBT. Store this solution for only a short period (overnight at the very most) at 4°C.
•Primary antibody	1:50 mouse anti-DLG monoclonal antibody and 1:2000 rabbit anti-HA polyclonal antibody in dilution buffer**.**
•Secondary antibody	1:250 biotinylated goat anti-rabbit and 1:200 Alexa-546 anti-mouse in dilution buffer.
•Florescence dye	1:500 Alexa Fluor 635 streptavidin in dilution buffer.

PROCEDURES

DISSECTION

•Anaesthetize adult flies on ice.

•Place flies onto a dissection dish and immerse them in PBS.

•Remove the head cuticle from the brain and clean the brain with gentle forceps manipulation under a dissecting microscope.

•Collect the dissected brains using a loop without touching the brain and place them in a 24-well plate with 100 ml PBS in each well on ice.

FIXATION

•The brain is placed in 4% paraformaldehye in PBS on ice and rapidly fixed with microwave irradiation for 90 s on a rotation plate, three times. **!CAUTION** Seal the well with a color tape to avoid dehydration.

•Keep the brain in blocking buffer at room temperature for 2 hours.

•Expel the air trapped in the tracheal system by keeping the sample in blocking buffer within a vacuum chamber (depressurize to -70 mmHg and keep it for 10 min), 4 cycles.

•Keep the brain in the blocking buffer in 4°C overnight.

IMMUNOHISTOCHEMISTRY

•Brain samples are washed with washing buffer for 30 min at room temperature, three times.

•Incubate with anti-DLG as well as anti-HA antibodies (120 µl/well) on an orbital shaker at 4°C for 2 days.

•Wash with washing buffer for >2 hrs at room temperature, three times.

•Keep the samples in washing buffer on an orbital shaker at 4°C for overnight.

•Incubate the samples with biotinylated anti-rabbit and Alexa-546 anti-mouse (120 µl/well) on an orbital shaker at 4°C for 2 days.

•Wash with washing buffer for >2 hrs at room temperature, three times.

•Incubate with Alexa Fluor 635 streptavidin on an orbital shaker at 4°C for overnight.

•Wash with washing buffer for >2 hrs at room temperature, three times.

•Clear the brains in *FocusClear*™ for 5 min, or until the brains become completely transparent at room temperature.

•Mount the brains in a drop of *MountClear*™ under a coverslip separated by a spacer ring of ~200 µm thickness, so that the brain is not flattened.

IMAGING

•Each image stack contains 120~160 optical sections with 0.32 x 0.32 x 1.0 m^3 voxel size taken under a 40X or 63X objective lens. Following settings were used: scanning speed 7, resolution 1,024 X 1,024 voxels, zoom 0.7, optical slice thickness 1 m, 50% overlap between two slices and averaged 4 times each scanning line.

•Two channels were simultaneously scanned: GFP-labeled neurons were excited using a 488 nm ArKr laser and Alexa Fluor 635-labeled pre-synaptic terminals were excited using a 633 nm HeNe laser. Alexa Fluor 546-labeled neuropilar structures were excited using a 543 nm HeNe laser

REFERENCE

Behavioral Genetics of the Fly (Drosophila melanogaster), ed. J. Dubnau. Published by Cambridge University Press.

CelExplorer

7F-4, No 246, Sec. 2, Guangfu Rd., East Dist.
Hsinchu 30068, Taiwan
Phone: 886-3-573-3330/ Fax: 886-3-573-3320

(URL) www.celexplorer.com