Protocol

Live Dissection and Surface Labeling of Proteins in *Drosophila* Embryos

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INTRODUCTION

This protocol presents methods for the specific detection of neuronal cell-surface receptors and secreted factors. These techniques allow visualization of surface pools of receptor proteins and also reveal the surface distribution of secreted cues. Live dissection of embryos to expose the ventral nerve cord is also described. This procedure can be used to improve the ability to detect signals from sub-optimal antibodies, where conventional staining gives poor results.

RELATED INFORMATION

The use of fluorescent and horseradish-peroxidase-conjugated antibodies to reveal axon-projection patterns and identify specific guidance molecules is described in **Visualizing Axons in the *Drosophila* Central Nervous System Using Immunohistochemistry and Immunofluorescence** (Bashaw 2010).

MATERIALS

CAUTIONS AND RECIPES: Please see Appendices for appropriate handling of materials marked with <**!**>, and recipes for reagents marked with <**R**>.

Reagents

Antibodies against proteins of interest
<**!**>Bleach (Clorox)
Blocking solution (PBS containing 5% normal goat serum)
Drosophila embryos
Glycerol (70% in PBS)
<**R**>Juice plates

Grape juice can be used as an alternative to apple juice in these plates.

Normal goat serum (GIBCO 16210-064)

Heat the goat serum for 30 min at 65°C. Divide into 5-mL aliquots and store at -20°C.

<**!**>Paraformaldehyde (4% in PBS)
<**R**>PBS (10X stock; pH 7.0)

Dilute to 1X prior to use.

Equipment

Coverslips (glass)
Double-sided sticky tape
Micropipettor (P1000) and tips
Microscope (dissecting)
Microscope slides (positively charged) (e.g., Fisher Scientific)

Embryos stick better to charged slides than they do to standard microscope slides.

Adapted from Drosophila *Neurobiology* (ed. Zhang et al.). CSHL Press, Cold Spring Harbor, NY, USA, 2010.
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Nytex mesh
PAP pen (for creating a hydrophobic boundary on the surface of a microscope slide)
Pasteur pipettes
Plastic probe (for transferring embryos from juice plate to microscope slide)

Use a 3-mL syringe and a plastic pipette tip. Melt the end of the pipette tip with a Bunsen burner to create a rounded, sealed plastic probe.

Squirt bottle
Tungsten dissecting needle
Vacuum aspirator

METHOD

Preparation of the Dissection Arena

1. Use a PAP pen to draw a boundary that conforms to the edges of a microscope slide.

   This hydrophobic boundary retains the staining solution and wash buffer.

2. Place a small piece of double-sided sticky tape (0.5 cm wide and 3 cm long) inside the area marked by the PAP pen. Ensure the tape is completely inside the boundary.

Collection of Embryos

3. Collect *Drosophila* embryos (for 20-24 h at 25°C) on small apple (or grape) juice plates.
4. Dechorionate the embryos by immersing them in 50% bleach for 3-5 min. Squirt the bleach directly onto the plate.
5. Strain the embryos through Nytex mesh and rinse with plenty of water.
6. Transfer the embryos to a fresh juice plate by gently blotting the mesh that contains the embryos onto the plate.

   Avoid transferring the embryos in large clumps, because this makes it more difficult to select the desired animals.

7. Under a dissecting microscope, select the desired stage of embryos and transfer them with a plastic probe to the sticky tape in the dissection arena (see Step 2). Transfer the embryos so that their anterior ends are pointing toward the top of the slide.

   Not all of the embryos are likely to make it through the entire procedure, so be sure to transfer more than you think you will need. To be sure to have 10-12 stained embryos at the end of the procedure, you will need to transfer ~20 embryos.

Dissection

8. Using a plastic probe or the blunt side of a tungsten dissecting needle, position the embryo ventral side down on the sticky tape. Gently roll the embryo, if necessary.
9. Using a P1000 pipette, cover the embryos with ~0.5 mL of PBS.
10. Under the dissecting microscope, gently insert the tungsten needle into the posterior third of the embryo and pierce the vitelline membrane.
11. With a smooth motion, push the needle toward the anterior end of the embryo.

    This should release the embryo from the vitelline membrane and the embryo should now be attached to your needle.

12. Place the embryo on the glass surface of the dissecting slide and push down gently with the needle to allow the embryo to adhere to the glass.

    Late-stage embryos (stage 16 or greater) do not stick well to the slide surface, so it is important to use embryos that are stage 15 or younger.

13. Repeat Steps 10-12 for each of the embryos on the sticky tape in the dissection arena.

14. Fillet each embryo by slicing down the dorsal midline with the needle and roll out the body wall until it makes contact with the slide.

15. Remove the guts and any other debris that obscures your view of the ventral nerve cord.

    The nerve cord is relatively thick and should be easily visible under the light microscope in embryos from stage 13 to stage 16.

Antibody Staining

It is important that the dissected embryos remain covered by buffer solution through all subsequent steps. Depending on the size of the dissection arena, use a volume between 0.5 mL and 1 mL. Exact volumes for wash steps are not important as long as the embryos remain covered by buffer solution. To change solutions, either use two pipettes to add and remove solutions simultaneously or use a P1000 pipette to add solution and a vacuum aspirator (set on low) mounted with either a Pasteur pipette or a P200 tip to remove the old solution as you add the new. Antibody concentrations are the same as for standard embryo staining (see ***Visualizing Axons in the*** **Drosophila** ***Central Nervous System Using Immunohistochemistry and Immunofluorescence*** *[Bashaw 2010]). Add the solutions gently or the embryos can be washed away!*

16. Replace the PBS used during dissection with fresh PBS.

17. Replace the PBS with blocking solution.

18. Incubate the slide for 15 min at 4°C.

19. Prepare primary antibody incubation as follows:

    i. Prepare 2 mL of the desired primary antibody diluted in PBS containing 5% normal goat serum chilled to 4°C.

    ii. Remove as much of the blocking solution as possible without uncovering the embryos.

    iii. Slowly add the antibody using a P1000 pipette until you have used the entire 2 mL. At the same time as you add antibody, you must also slowly remove antibody solution with the vacuum aspirator or second pipette.

    iv. Leave ~0.5 mL of primary antibody solution on the embryos.

20. Incubate in primary antibody for 30 min at 4°C.

21. Wash with 5 mL of cold PBS to remove the primary antibody.

    This should take 2-3 min with constant addition and removal of the PBS until the 5 mL is depleted.

22. Fix the embryos with 4% paraformaldehyde in PBS for 15 min at 4°C.

23. Wash with 5 mL of cold PBS.

    The following steps can be performed at room temperature.

24. Incubate in secondary antibody diluted in PBS containing 5% goat serum.

25. Wash with 5 mL of PBS. Leave for 5 min.

26. Repeat the wash.

27. Remove as much of the wash solution as possible and place a small drop of 70% glycerol in PBS over the embryos.

28. Cover with an appropriate sized glass coverslip.

    The embryos are now ready for viewing.

    When performing surface staining experiments, always include a control to ensure that the embryos have not been made permeable and that primary antibodies have not been internalized. Antibodies against known cytoplasmic or nuclear proteins can be used for this purpose.

REFERENCES

Bashaw GJ. 2010. Visualizing axons in the *Drosophila* central nervous system using immunohistochemistry and immunofluorescence. *Cold Spring Harb Protoc* doi: 10.1101/pdb.prot5503.

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