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Protocol

Antibody Staining of *Parhyale hawaiensis* Embryos

E. Jay Rehm, Roberta L. Hannibal, R. Crystal Chaw, Mario A. Vargas-Vila, and Nipam H. Patel[1]

Department of Molecular and Cell Biology, University of California, Berkeley, CA 94720-3140, USA
Department of Integrative Biology, University of California, Berkeley, CA 94720-3140, USA
Howard Hughes Medical Institute, University of California, Berkeley, CA 94720-3140, USA

INTRODUCTION

The great diversity of arthropod body plans, together with our detailed understanding of fruit fly development, makes arthropods a premier taxon for examining the evolutionary diversification of developmental patterns and hence the diversity of extant life. Crustaceans, in particular, show a remarkable range of morphologies and provide a useful outgroup to the insects. The amphipod crustacean *Parhyale hawaiensis* is becoming established as a model organism for developmental studies within the arthropods. This protocol provides a simplified protocol for antibody staining of *P. hawaiensis* embryos. The method also works well for other arthropods and phyla. Fixed embryos are rehydrated, washed, blocked with normal goat serum, and incubated overnight with primary antibody. Embryos are then washed and incubated with a peroxidase-conjugated secondary antibody that binds to the primary antibody. A subsequent histochemical reaction produces a black stain in those cells where antibodies have localized.

RELATED INFORMATION

The fixed embryos used in this protocol were prepared as described in **Fixation and Dissection of *Parhyale hawaiensis* Embryos** (Rehm et al. 2009a). These embryos are also suitable for in situ hybridization to probe mRNA expression, as in **In Situ Hybridization of Labeled RNA Probes to Fixed *Parhyale hawaiensis* Embryos** (Rehm et al. 2009b). Cell-lineage analysis can be performed following injection of *P. hawaiensis* embryos with fluorescent tracers, which is detailed in **Injection of *Parhyale hawaiensis* Blastomeres with Fluorescently Labeled Tracers** (Rehm et al. 2009c). An introduction to *P. hawaiensis* that discusses husbandry, the developmental stages of this crustacean, and its utility as a model organism for studies of arthropod development and evolution is presented in **The Crustacean *Parhyale hawaiensis*: A New Model for Arthropod Development** (Rehm et al. 2009d).

MATERIALS

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

Reagents

<**R**>DAB + Ni
<**R**>DAPI solution

[1]Corresponding author (nipam@uclink.berkeley.edu)
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<**R**>Glycerol solutions (50% and 70%)
<**!**>H_2O_2 (hydrogen peroxide) (3%)
<**!**>Methanol (70%, 50%, and 30%; all dilutions in PT)
P. hawaiensis embryos

This protocol assumes that the P. hawaiensis *embryos have been fixed for 15-20 min and dehydrated for storage in 100% methanol as described in* ***Fixation and Dissection of* Parhyale hawaiensis *Embryos*** *(Rehm et al. 2009a).* D. melanogaster *embryos are often included in the same tubes as internal controls and to help reduce overall background. Prepare large volumes of dechorionated, fixed, and dehydrated* D. melanogaster *embryos, and store them at -20°C (Patel 1994) for this purpose.*

<**R**>PBS(P) (1X)
Primary antibody
<**R**>PT
<**R**>PT + NGS
Secondary antibody, conjugated with horseradish peroxidase (HRP)

Equipment

Dissection microscope
Light source (see Step 1)
Microcentrifuge tubes
Micropipettor with tips
Pipettes (glass)
Test tube rack for microcentrifuge tubes

METHOD

Perform all incubations and washes in microcentrifuge tubes at room temperature.

Rehydration of Embryos

1. Rehydrate *P. hawaiensis* embryos stored in 100% methanol by incubating them for 5 min each in 70%, 50%, and 30% methanol. During rehydration and all subsequent wash steps, gently mix the embryos by spinning the microcentrifuge tubes as they rest in a tube rack. When changing solutions, pipette away as much as possible without allowing the embryos to dry. Avoid shaking or flicking the tubes because the embryos will splash up the tube sides, dry out, and not stain properly.

   P. hawaiensis *embryos often sink slowly (or not at all) to the bottom of the tube. Be careful to avoid pipetting away these embryos when changing solutions. It is often necessary to illuminate the tube with a strong localized light source (such as that provided by a typical fiber optic lamp designed for a dissection microscope) to ensure that the translucent embryos are not inadvertently withdrawn. Glass pipettes can be used to change solutions, but embryos will stick to glass.*

   See Troubleshooting.

   Fly embryos in methanol require only a series of 100% PT washes (three times for 5 min) for rehydration.

Primary Antibody Reaction

2. Wash the *P. hawaiensis* and fly embryos with PT three times for 5 min. Following the washes, add ~10 µL of fly embryos to each tube of *P. hawaiensis* embryos.
3. Incubate the embryos in 300 µL of PT + NGS for 30 min at room temperature to block nonspecific antibody-binding sites.
4. Add the appropriate amount of primary antibody to achieve the desired final concentration.
5. Gently swirl the embryos in the antibody solution and incubate overnight at 4°C.

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Secondary Antibody Reaction

6. Wash the embryos with PT three times for 1 min.

   Before the washes, diluted primary antibody can be recovered for reuse. Store diluted antibody at 4°C. It can typically be reused several times.

7. Wash the embryos with PT three times for 30 min.
8. Incubate the embryos in 300 µL of PT + NGS for 10-30 min at room temperature.
9. Add an appropriate HRP-conjugated secondary antibody to the proper final concentration.
10. Gently swirl the embryos in the secondary antibody solution and incubate for 2 h at room temperature.
11. Wash the embryos with PT three times for 1 min.
12. Wash the embryos with PT three times for 30 min.

Histochemical Development Reactions

The following section describes a histochemical reaction for use with secondary antibodies that have been conjugated with HRP. The colorless substrate DAB becomes brown when oxidized by the free oxygen that is formed by the action of peroxidase on the hydrogen peroxide. The presence of nickel ions results in a darker purplish-black. Staining the embryos with DAPI following the HRP reaction enables the nuclei of all cells to be visualized in the ultraviolet spectrum. Moving reacted embryos into 70% glycerol increases the optical clarity of the tissue and makes it easier to mount.

13. Prepare DAB + Ni solution immediately before use.

    1 mL of DAB is sufficient for three reactions.

14. Pipette away the final wash from Step 12 to within 1 mm of the embryos.
15. Add 300 µL of DAB + Ni. Mix very gently, ensuring that no bubbles are present.
16. Prepare a 0.3% solution of H_2O_2 by mixing 10 µL of 3% H_2O_2 with 90 µL of 1X PBS(P).

    This solution will last only ~30 min.

17. Add 15 µL of 0.3% hydrogen peroxide to each tube of embryos in DAB + Ni. Mix. Swirl the tubes gently, mixing the reagents quickly and thoroughly. Avoid introducing bubbles. Remove any bubbles that appear.

    The addition of H_2O_2 initiates the reaction. At this point, monitor the reaction by opening the lid of the microcentrifuge tube and observing the embryos under a dissection microscope. Allow the reaction to proceed until the signal-to-noise ratio is satisfactory. Reactions typically continue for 5-10 min.

18. Stop the reaction by removing the DAB and washing several times with PT.

    See Troubleshooting.

19. If DAPI staining is desired, place the reacted embryos into 200 µL of DAPI solution for 30 min or longer. If no DAPI staining is required, simply put the embryos into 50% glycerol for 30 min.
20. Move the embryos to 70% glycerol.

    Embryos can be stored in 70% glycerol for several weeks at room temperature, several years at 4°C, and several decades at -20°C.

TROUBLESHOOTING

Problem: Embryos stick readily and permanently to the inner surfaces of glass pipettes.
[Step 1]
Solution: Consider the following:

1. Pass yolk from dissected embryos into pipettes that will be used in proximity to the embryos, thus coating the pipettes with a nonstick layer of yolk proteins.
2. Alternatively, rinse pipettes with PT before using them near embryos.

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Problem: There is a weak signal (understaining).
[Step 18]
Solution: There are several possible causes of a weak signal. The most common in *P. hawaiensis* are stopping the staining reaction too soon and overfixation. Consider the following:

1. If the signal remains weak after 10 min of reaction, add another 15 µL of 0.3% hydrogen peroxide to the tube. Monitor the reaction closely because it may react quickly and result in overstaining.
2. Make sure that specimens are fixed for no longer than 15-20 min.

Problem: There is high background (overstaining).
[Step 18]
Solution: The most common reasons for high background in *P. hawaiensis* are underfixation of the tissue or the fact that the reactions were allowed to proceed for too long. Consider the following:

1. Be sure to prepare the DAB + Ni solution just before use.
2. Reduce excess background by washing the embryos overnight in PT before placing them in glycerol.

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REFERENCES

Patel, N.H. 1994. Imaging neuronal subsets and other cell types in whole mount *Drosophila* embryos and larvae using antibody probes. *Methods Cell Biol.* **44:** 445–487.

Rehm, E.J., Hannibal, R.L., Chaw, R.C., Vargas-Vila, M.A., and Patel, N.H. 2009a. Fixation and dissection of *Parhyale hawaiensis* embryos. *Cold Spring Harb. Protoc.* (this issue). doi: 10.1101/pdb.prot5127.

Rehm, E.J., Hannibal, R.L., Chaw, R.C., Vargas-Vila, M.A., and Patel, N.H. 2009b. In situ hybridization of labeled RNA probes to fixed *Parhyale hawaiensis* embryos. *Cold Spring Harb. Protoc.* (this issue). doi: 10.1101/pdb.prot5130.

Rehm, E.J., Hannibal, R.L., Chaw, R.C., Vargas-Vila, M.A., and Patel, N.H. 2009c. Injection of *Parhyale hawaiensis* blastomeres with fluorescently labeled tracers. *Cold Spring Harb. Protoc.* (this issue). doi: 10.1101/pdb.prot5128.

Rehm, E.J., Hannibal, R.L., Chaw, R.C., Vargas-Vila, M.A., and Patel, N.H. 2009d. The crustacean *Parhyale hawaiensis*: A new model for arthropod development. *Cold Spring Harb. Protoc.* (this issue). doi: 10.1101/pdb.emo114.