

*Gryllus* Techniques and Protocols**Antibody Staining (Immunohistochemistry)**

*To visualize gene expression in cricket embryos*

General Notes:

- Embryos are kept in 1.5mL eppendorf tubes.
- All washes are 5 minutes, room temperature and in ~1.4mL solution unless otherwise stated. Swirl to mix after adding solution unless otherwise stated.
- Be careful not to expose embryos to air when removing washes.
- Pipette solution on side of tube to avoid harshly disrupting embryos.
- Throughout, be careful not to pipette up or destroy embryos.

Day 1:

- 1) Rehydrate embryos that have been stored in methanol. Either leave washes on a rotating tube rack or swirl to ensure appropriate mixing.
  - a. 75% MeOH in PBSTw
  - b. 50% MeOH in PBSTw
  - c. 25% MeOH in PBSTw
- 2) Wash 3x in PBSTw. Either leave washes on a rotating tube rack or swirl to ensure appropriate mixing.
- 3) Block in 300µl of 5% NGS/PBSTw for 90min.
- 4) Incubate in your primary antibody mix at a manufacturer suggested concentration, overnight at 4°C.

Day 2:

- 1) Wash 3x in PBSTw for 1 minute. After each wash, swirl, let embryos collect in the bottom of tube, and then change wash.
- 2) Wash 6x in PBSTw for 60 minutes.
- 3) Block in 300µl of 5% NGS/PBSTw for 90min.
- 4) Incubate in your secondary antibody mix and a nuclear stain at a manufacturer suggested concentration, overnight at 4°C. Cover with tinfoil to avoid photobleaching. Typically, we have found 1:1,000 dilution for all the Alexa-coupled secondary antibodies and 1:5,000-1:10,000 for Hoeschst works well.

Day 3:

- Embryos should be kept covered with tinfoil from the secondary antibody incubate forward. You can remove tinfoil to change wash solution, but keep covered during washes.

- 1) Wash 3x in PBSTw for 1 minute. After each wash, swirl, let embryos collect in the bottom of tube, and then change wash.
- 2) Wash 6x in PBSTw for 60 minutes.
- 3) Post-fix in 4% formaldehyde in PBSTw for 30 minutes.
- 4) Wash 3x in PBSTw for 1 minute. After each wash, swirl, let embryos collect in the bottom of tube, and then change wash.
- 5) Wash 3x in PBSTw.
- 6) Store embryos in 50µl of Vector Laboratories Vectashield Mounting Medium, at 4°C. Once mounted, embryos on slides can be stored in colder temperatures.

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Recipes

*PBS:*

Add 2.05g  $\text{NaH}_2\text{PO}_4\text{-H}_2\text{O}$  and 11.98g  $\text{NaHPO}$  to 800mL of DEPC- $\text{H}_2\text{O}$ .

Measure pH. If pH is not 7.4 +/-0.4 adjust with HCl or NaOH.

Add 81.76g NaCl

*PBST<sub>w</sub>:*

Dilute 50mL 10X PBS to 1X with 895mL DEPC- $\text{H}_2\text{O}$ .

Autoclave and cool.

Add 5mL 20% Tween-20