Antibody staining of whole mount Drosophila embryos

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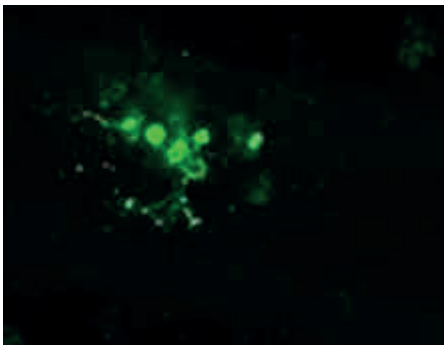
Protocol for antibody staining of whole mount Drosophila embryos using GFP antibody (ab290)



IF image produced using Bossing's whole mount protocol

Embryos were injected with a plasmid carrying a fusion of the Actin coding region to GFP (Abcam) ab290

(Bossing et al., 2002). Expression is activated by the GAL4/ UAS system (Brand and Perrimon, 1993).

Image courtesy of Dr. T Bossing.

Protocol:

1. Prepare fixation mix in 1.5 ml cap: 400 µl PBS, 100 µl 40% formaldehyde and 500 µl n-heptane. For the detection of some extracellular antigens it helps to add 0.02% SDS or to fix the embryos in 500 µl picric acid / 500 µl n-heptane. Vortex the fixation mix at highest speed for 1 min.

2. Remove yeast and dechorionate embryos by covering the apple-juice plate with 100% bleach for 2min; slight rotations of the apple-juice plate will bring the dechorionated embryos to the surface.

3. During bleaching: close the narrow opening of a funnel with a nylon-mesh (diameter of holes: 40 µm), attach mesh by wrapping a rubber band around the funnel.

4. Wash embryos into the funnel by squirting deionized water over the plate, especially along the edges of the plate; wash embryos in funnel by squirting water along the funnel three times.

5. Remove rubber band and mesh; pick up the embryos with a very fine paint brush; dechorionated embryos will adhere to the brush; dip brush with embryos into the fixation mix and whisk the brush.

6. With the cap lying on its side fix embryos for 15 min on a shaker with gentle rotation.

7. During fixation: heat the tip of a glass Pasteur pipette over a Bunsen burner; aim the flame at the middle of the tip and hold the end of the tip with two fingers *(glass is a bad heat conductor therefore your fingers are safe)*; when the middle glows red remove the tip from the flame and immediately pull; break away the long drawn out and very thin part from the tip. As an alternative to the glass pipette it is possible to use a 1 ml Gilson pipette.

8. To stop fixation remove about 80% of the lower phase of the fixation mix with the pipette; fill up the cap with 1 ml of methanol and vortex on highest speed for about 1 min. This procedure will remove the extraembryonic membrane.

9. 2 min after vortexing the majority of the embryos will be at the bottom of the cap. If not all embryos sink to the bottom of the cap, remove as much of the liquid as possible and refill the cap with methanol. Do not vortex again. After most of the embryos are at the bottom of the cap, remove all liquid and wash embryos three times with methanol.

10. Rehydrate the embryos with three washes in PBT (PBS with 0.3% Triton added); incubate in 20% newborn calf serum / PBT on shaker for at least 10 min. Note that Triton only slowly dissolves in PBS i.e. set up the solution about 20 min in advance. PBT can be kept at room temperature indefinitely. Calf serum should be stored in the fridge and 0.02% sodium azide should be added.

11. Add primary antibody diluted in 10% NCS / PBT and 0.02% sodium azide; most primary antibodies can be used at least three times i.e. store the primary after the first incubation at 4°C for further stainings; diluted 1:1000 (Abcam) ab290 can be re-used five times.

12. Depending on the quality of the primary antibody, incubations can last from 2 hr at room temperature (high affinity antibodies) to overnight at 4°C (low affinity antibodies). Overnight incubation at 4°C aides the perfusion of the antibody. For (Abcam) ab290 2h at room temperature are sufficient.

13. Wash procedure: three rinses with PBT, 10min incubation with 30% NCS / PBT, three rinses with PBT, 10min incubation with 30% NCS / PBT, three rinses with PBT.

14. Add secondary antibody diluted in 10% NCS / PBT for 2 hr at room temperature, lay cap on its side on a gently moving shaker.

15. Repeat step 13.

16. Non-fluorescent detection: use Diaminobenzidine assay (normal signal) or Alkaline Phosphatase assay (weak signals); develop Alkaline phosphatase signal in the dark and take an aliquot of embryos out of the cap with a 1ml Gilson pipette to follow the staining; postfix embryos with 4% formaldehyde / PBT for 10 min to stabilize Alkaline Phosphatase signal.
    Enhancement of very weak signals: use Vectastain ABC Elite Kit, or TSA Kit
    Fluorescent detection: use secondary antibodies coupled to Alexa488, Alexa568 or Cy5

17. Incubate embryos in 50% glycerol / PBS until they sink to the bottom of the cap (about 10 min); replace the 50% glycerol I /PBS with 70% glycerol / PBS wait again until embryos are at the bottom of the cap (about 1hr); replace 70% glycerol/ PBS with 90% glycerol / PBS and allow embryos to sink to the bottom of the cap. Embryos can be stored in 90% glycerol / PBS at 4°C for at least three years. There is no need for any light protection for fluorescent stainings.