

## Antibody staining of larval eye discs

1. Dissect uncrowded, well-fed 3<sup>rd</sup> instar larvae in 1X PBS.
2. Keep eye discs in PBS, on ice, until ready to fix (no longer than one hr).
3. Place eye discs in the fixative (Formalin- 37% formaldehyde solution diluted in PBS 1:5). Fix for 30 min at RT with gentle rocking.
4. Wash 4x 8-10 mins with PBST (1X PBS and Triton X 100 at 0.3 %) with gentle rocking.
5. Block for 30 min with PBST + NGS (normal goat serum) 10% solution. Use fresh blocking solution. Do not save for more than 2-3 days.
6. Dilute primary antibody to appropriate concentration in blocking solution and add 8-10 microlitres to single wells of a micortiter plate.
7. Using forceps grasp mouth hooks, place eye discs (1-3 pairs) in individual wells containing primary antibody. Incubate overnight at 4C.
8. Wash 4x 8-10 mins with PBST in pyrex well dishes.
9. Block for 30 min as in step 5.
10. Dilute secondary antibody to appropriate concentration in blocking solution. Use about 200 microliters for each well of the pyrex well dishes.
11. Add secondary antibody to eye discs in well dishes and incubate for 2 hrs at RT with gentle rocking. Cover with a box to prevent the secondary antibody signal from bleaching away.
12. Wash 4x 8-10 mins with PBST with gentle rocking.
13. Mount in vectashield.

### In case of HRP-Ab

9. Wash in PBST x 6 times, PBS x 1.
10. Color reaction: Put samples in 0.5 mg/ml of DAB (diluted 1:2 in PBS from 2X stock) + 5 ul 8% NiCl<sub>2</sub>/ml solution (forms black precipitates). Add 5ul/ml of 3% H<sub>2</sub>O<sub>2</sub>. Watch colour reaction (<10 min) probably even (<5 min).
11. Stop the reaction in PBS.
12. Mount in glycerol if only HRP conjugated antibody is used and seal.