

Date: \_\_\_\_\_

# Antibody Staining Protocol

Purpose of experiment:

1. Dissect eye discs in 1XPBS (no longer than 30' for any one batch).

<u>Sample #/color</u>	<u>Genotype</u>	<u># discs</u>	<u>1° Ab</u>	<u>dilution</u>	<u>2° Ab</u>	<u>dilution</u>
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- 2a. Fix for 30' in PLP.

- 2b. (optional) Remove peripodial membrane.

3. Wash 3 x 5' in 1XPBS.

4. Incubate in PBST (PBST = 1XPBS + 0.4% Triton X) + 10% goat serum for 40'.

5. Incubate in 1° at 4° C overnight.

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6. Wash 12 X 10' in PBST.

7. Incubate in PBST (PBST = 1XPBS + 0.4% Triton X) + 10% goat serum for 40'.

8. Incubate in 2° antibody at room temp. for 2 hours.

9. Wash 6X in PBST, 6X in PBS.

10. Color reaction: put samples in 0.5 mg/ml DAB (diluted 1:2 in PBS from 2X stock) + 5 µl 8% NiCl/ml solution. Add 10 µl of 30% H<sub>2</sub>O<sub>2</sub>/well. Watch color reaction (shouldn't take longer than 10').

11. Stop reaction in 1XPBS.

12. Mount in GOOD glycerol; seal with nailpolish.

Results: