

Date: _____

Antibody Staining Protocol

1. Dissect eye discs in 1XPBS (no longer than 30' for any one batch).
2. Fix for 30' in PLP.
3. Wash 3 x 5' in 1XPBT (PBT = 1XPBS + 0.4% Triton X)
4. Incubate in PBST (PBST= PBT + 10% goat serum) for 40'.
5. Incubate in 1° at 4° C overnight in PBT + Goat Serum.
6. Wash 5 X 10' in PBST.
7. Incubate in PBT + 10% goat serum for 40'.
8. Incubate in 2° antibody at room temp. for 2 hours.
9. Wash 6X in PBT
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% H₂O₂/well.
11. Stop reaction in 1XPBS.
12. Mount in GOOD glycerol; seal with nailpolish.