Antibody staining of *Drosophila* embryos

Begin with embryos in 100% MeOH (stored at -20°C or freshly fixed).

1. Transfer 50-100ul embryos to a labeled 1.5ml microcentrifuge tube. (Label with cross or stock genotype(s), antibodies used and date. Record this information in your lab notebook.)
2. Rehydrate embryos by rinsing 3x with 1ml PBT, then washing 2x 5min with 1ml PBT.
3. Block embryos in PBT+5% NGS. Incubate on nutator at room temp for at least 30min.
4. Add 1° antibody, diluted in PBT+5% NGS. 500ul per tube (1ml per tube can be used if staining a larger volume of embryos).
5. Incubate O/N on nutator in cold room (4°C).
6. Rinse 3x with 1ml PBT, then wash 2x 5min with 1ml PBT.
7. Add 2° antibody, diluted in PBT+5% NGS. 500ul per tube.
8. Incubate at least 1 hr on nutator at room temp.
9. Rinse 3x with 1ml PBT, then wash 2x 5min with 1ml PBT.

for HRP-conjugated antibody staining, proceed to step 10.
for fluorescent antibody staining, skip steps 10-13 and proceed directly to step 14.

10. Add 500ul Stable DAB (Invitrogen 750118; pre-warmed to room temp) per tube.
11. Incubate 5min on nutator at room temp.
12. Remove DAB and dispose in “DAB waste” vessel in fume hood.
13. Rinse 3x with 1ml PBT.

14. Rinse with 1ml PBS.
15. Resuspend by gentle vortexing in at least 500ul 70% glycerol/PBS.

Store stained embryos in a cardboard freezer box at room temp or 4°C. Protect from light! Some antibody/fluorophore combinations will remain discernible for a year or more; others will fade in a few months. Best practice is to dissect/score/image fluorescently stained embryos as soon as possible after staining. DAB-developed embryos can be stored for years without loss of staining.

PBT (1xPBS with 0.1% Triton X-100) recipe:

100 ml 10X PBS
5 ml 20% Triton X-100
H_2O to 1 L