DAB Staining

-Embryos should be bleached prior to staining to quench endogenous peroxidase activity
-Use of MetOH decreases bubbling, which can damage embryos

Day One-

1. Wash embryos in 100% MetOH for at least 5 minutes.
2. Wash 2 X 5' in PBS
3. Bleach 20'-2 hours under fluorescent light (bleach soln; PBS + 1% Hydrogen Peroxide + 5% Formamide)
4. Wash 15' in PBT (PBS + 0.1% Triton, Triton [ ] can be increased)
5. Block 1 hour at room temp in PBT + 10% HIGS
6. Incubate o/n at 4°C in 1° Ab diluted in PBT + 5% HIGS

Day Two-

1. Wash 3 x 2 hours, plus several short washes in PBT
2. Incubate o/n at 4°C in 2° Ab diluted in PBT + 5% HIGS

Day Three-

1. Wash at least 3 x 1 hour in PBT
2. Wash 1 x 5' in PBS
3. Incubate in DAB solution until staining is sufficient (usually 1'-5').
4. Wash several times in PBS
5. Wash and store in 70% EtOH at room temp or 100& EtOH at -20°C.

DAB Solution (Sigma D-4168 or D-4293):

With large tablets-dissolve 1 DAB tablet in 5ml PBS, add Hydrogen Peroxide to 0.5µl/ml
OR
With small tablets dissolve 1 DAB and 1 Hydrogen Peroxide tablet in 1ml water.