BrdU protocol for eye discs (or any discs)

1) Dissect discs in Schneider's. Incubate 30-60' in Schneider's + 75µg/ml BrdU. BrdU stock is made fresh each time in 80% EtOH as a 7.5 mg/ml solution. **Retain larval carcasses for antibody preabsorption: fix, wash and freeze in PBT.**

2) Wash 2X5' in Schneider's, 1X5' in PBS. Transfer tissue to a basket made from fine Nitex mesh and a Sarsdedt screw-cap tube. All incubations are done in 2 ml volumes in a 24-well plate.

3) Fix 15' in 4% formaldehyde in PBS (diluted from 37%). Fix 15' in 4% formaldehyde in PBS + 0.6% Triton X-100. Wash 2X10' in PBS + 0.3% Triton X-100.

BrdU staining protocol:

4) Permeabilize 30-60 min. in PBS + 0.6% Triton X-100.

5) Transfer to 1:1 mixture of PBS + 0.6% Triton X-100:4 N HCl (2 N final conc.) for 30 min.

6) Wash 3X10 min. in PBS + 0.3% Triton X-100.

7) Incubate overnight in a 1:100 dilution of α-BrdU (Beckton-Dickinson) in PBS + 0.3% Triton X-100 + 10% goat serum.

8) Wash 3X10 min. in PBS + 0.3% Triton X-100.

9) Incubate in 2˚: Bio-Rad goat α-mouse, 1:100 in PBS + 0.3% Triton X-100.

10) Wash 1X10 min. in PBS + 0.3% Triton X-100, 2X10 min. in PBS.

11) Post-fix 10 min. in 1% glutaraldehyde in PBS; wash 3X10 min. in PBS.

12) Perform DAB reaction as usual.