

## Whole mount Oil Red O Staining for Zebrafish Embryos

### Fixation:

1. Fix embryos in 4% paraformaldehyde: 2-4 hours @ RT.
2. Wash – 3X 2' with PBS (NOT PBST!)
3. Store in PBS at 4 C for up to 1 month.

### Staining:

1. Wash embryos with PBS – 2 X 1'.
2. Transfer to Corning NetWells for 12 well dish. May need to use PBS with 0.05% tween to prevent sticking. If tween is used, minimize the time in the tween and wash Tween out with PBS.

3. If embryos were not treated with PTU, pigment must be removed by bleaching. Note that this will introduce bubbles to the embryos, and this makes them difficult to photograph.

Bleach solution: (can be stored for ~ 1 week at 4°C. If stored, treat for longer with bleach).

6 mls H<sub>2</sub>O  
0.25 mls 20X SSC  
0.5 mls formamide  
3.3 mls H<sub>2</sub>O<sub>2</sub>

Bleach day 5 embryos for 20 minutes at RT (15-18 mins for day 4, 10 mins for day 3 and ~5-8 mins for day 2).

4. Wash 5X with PBS, quick washes.
5. 85% Propylene glycol: 10 minutes.  
100% Prop. glycol: 10 minutes. Drain thoroughly before putting in oil red O.  
Save for day 2 washes.

6. Oil Red O in 100% prop. glycol (polysciences): over night at room temperature, rocking gently.

7. 100% prop. glycol: 30'  
85% prop. glycol: 50'  
85% prop. glycol: 40'

8. While embryos are in 2<sup>nd</sup> 85% PG wash, add equal amount of PBS and swirl to mix – 5' at RT.

9. Transfer to PBS 15' then 5' in PBS.

10. Transfer from Netwells back to tubes using some PBS-0.05% tween if necessary to avoid embryos sticking to transfer pipettes.

11. Wash 1X with PBS – quick.
12. Add ~300  $\mu$ l 80% glycerol, store at RT indefinitely.