

**OCT embedding of B-Galactosidase Stained Whole Embryos
(modified protocol from Krumlauf Lab and Jing Yu of McMahon Lab)**

1. Wash embryos in 1X PBS, pH 7.4.
2. Prepare 20ml of fixative (1% formaldehyde, 0.2% glutaraldehyde, 2mM MgCl₂, 5mM EGTA, 0.02% NP-40) as follows:

37% formaldehyde	540µl
25% glutaraldehyde	160µl
1M MgCl ₂	40µl
0.5M EGTA	200µl
2% NP-40	200µl
Q.S. to 20ml with ddH ₂ O	18.86ml

3. Fix embryos as follows:
60-90min for E13.5 and later
30-60 min for E12.5 and earlier
4. Wash embryos 3 x 15min with 1X PBS + 0.02% NP-40 to permeabilize.
5. Prepare 12ml of stain solution (5mM K₃Fe(CN)₆, 5mM K₄Fe(CN)₆, 2mM MgCl₂, 0.01% Na deoxycholate, 0.02% NP-40, 1mg/ml X-gal) as follows:

0.5M K ₃ Fe(CN) ₆	120µl
0.5M K ₄ Fe(CN) ₆	120µl
1M MgCl ₂	24µl
1% Na deoxycholate	120µl
2% NP-40	120µl
50mg/ml X-gal	240µl
Q.S. to 12ml with 1X PBS, pH 7.4	11.256ml

6. Stain for 30min to O/N at 37°C
7. Wash embryos 3 x 15min at 4°C with 1X PBS, pH7.4.
8. Post-fix sample in 4% paraformaldehyde for 20min at RT.
9. Wash 3 x 5min at RT with 1X PBS, pH 7.4.
10. Wash 1 x O/N at 4°C with 1X PBS, pH 7.4.
11. Store/photograph embryos in 1X PBS + 80% glycerol.