Lac Z staining

Modified protocol from Krumlauf Lab.
Also see Methods in Enzymology 225:451-46C-X Tong, B. Chubak

1. Wash the embryos – organs in PBS supplemented with Mg\(^2\)+&Ca\(^2\)+ (final conc. 0.5mM and 1mM respectfully) (Dulbecco’s PBS Cellgro; Cat # 21-030-CV)
   - 500ml PBS- add 500µl of 1M CaCl\(_2\)
   - add 250µl of 1M MgCl\(_2\)

2. Fix Embryos – organs: 10-20 min for < 11.5dpc; 30min for>11.5dpc (even 1-2hours)

**Glutaraldehyde Fixative**

Glutaraldehyde Buffer
- 5mM EGTA
- 2mM MgCl\(_2\)
- 0.1M NaPi pH 7.3
- store @ RT

Add fresh:
- **A.** Glutaraldehyde to 0.1% (v/v) from 50% stock (kept at +4°C) use the fume hood
- **B.** Formaldehyde to 1.5% (v/v) from 37% stock (kept @RT, Laina’s closet)

50ml Fixir: 50µl 50% glutaraldehyde
- 750µl 37% Formaldehyde

3. Wash 3X30min in PBS/0.02% NP-40 (IGEPAL CA-630; Sigma)

4. Stain for 90 min to ON @ 30°C, or @ 22°C if performing RNA in situ double labeling. Staining solution should be made fresh, can be stored up to 7 days @ +4°C

<table>
<thead>
<tr>
<th>Solution</th>
<th>12ml total volume</th>
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</thead>
<tbody>
<tr>
<td>5mM K(_3)Fe(CN)(_6)</td>
<td>0.120ml of 0.5M stock</td>
</tr>
<tr>
<td>5mM K(_4)Fe(CN)(_6)</td>
<td>0.120ml of 0.5M stock</td>
</tr>
<tr>
<td>2mM MgCl(_2)</td>
<td>0.024ml of 1M stock</td>
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<tr>
<td>0.01% NaDeoxycolate</td>
<td>0.120ml of 1% stock</td>
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<tr>
<td>0.02% NP-40</td>
<td>0.120ml of 2% stock</td>
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<tr>
<td>1 mg/ml X-gal</td>
<td>0.240ml 50mg/ml stock</td>
</tr>
<tr>
<td>PBS</td>
<td>Up to 12ml</td>
</tr>
</tbody>
</table>
Notes:
- Use 0.04% of NaDeoxycolate for adequate penetration after 14.5dpc

5. Wash 3X with PBS
6. Post-fix in 4% paraformaldehyde in PBS /ON
7. Store/photograph in PBS and 0.1% paraformaldehyde (PFA)

Reagents:
X-gal; Roche Cat#651 745. resuspend the powder in N,N-Dimethylformamide (Sigma cat#D-4254) to 50mg/ml
EGTA- Ethylene glycol-bis (β aminoethyl ether)-N,N,N',N'-tetraacetic acid tetrasodium salt, SIGMA E8145
K₄Fe(CN)₆- Potassium hexacyanoferrate (II) trihydrate; SIGMA P9387
K₃Fe(CN)₆- Potassium hexacyanoferrate (III)- Potassium ferricyanide; SIGMA P3667
Sodium deoxycolate-Deoxycolic acid C₂₄H₃₉O₄Na; SIGMA D6750
Glutaraldehyde solution 25% in water, Grade II; SIGMA G6257

Dehydration

1) Wash the tissues in PBS (~10 min)
2) 50% EtOH for 20-30 min
3) 70% EtOH for 20-30 min
4) 80% EtOH for 20-30 min
5) 90% EtOH for 20-30 min
6) 95% EtOH for 20-30 min
7) 100% EtOH for 20-30 min, 2X
8) isopropanol for 20-30 min, 2X
9) paraffin @ 60°C for 30 min
10) embed

Make sections and then contrastain with eosin

Dewaxing

1) isopropanol or histoclear for 5 min, 3X
2) 100% EtOH for 1 min, 2X
3) 95% EtOH for 1 min
4) 70% EtOH for 1 min

Staining

5) eosin 1-5min
Dehydration

6) 70% EtOH for 1 min
7) 95% EtOH for 1 min
8) 100% EtOH for 1 min, 3X
9) isopropanol or histoclear for 1 min, 2X
10) xylene 1’
11) Mounting in Permount (xylene based)