Alcian green stain for cartilage  (→ chick limb development)

Alcian green stain binds to cartilage, but not calcified bone. This allows studying the early development in limbs and noting any differences in structure formation.

1. Remove limbs from host choioallantoic membrane and fix in 10 % formalin in PBS for 1-2 h at room temp (→ in fume hood!!).

2. Wash 3 times with PBS (20-30 min each; tissue can be stored in PBS at 4 C).

3. Immerse in Alcian green stain for 4 hours with gentle agitation.

4. Wash 3 times with 70% ethanol.

5. Wash with 85%, 95%, and 100% ethanol.

6. Clear limbs by washing 1-3 times with methyl salicylate.

Solutions:

Alcian green stain (100 ml)

0.1g  Alcian green
70 ml  100% ethanol
1 ml  conc. HCl
29 ml  d. d. H2O

PBS = phosphate-buffered saline
10 X PBS stock

NaH2PO4  0.222 g
Na2HPO4  1.190 g
NaCl   8.770 g
KCI  0.200 g
→ dissolve in 90 ml d.d. H2O
→ adjust pH to 7.4 (using HCl or NaOH)
→ adjust volume to 100 ml with d.d. H2O; store at 4 C.

PBS
Mix 1 part 10 X PBS with 9 parts d.d. H2O; adjust pH to 7.4.
Fixative (mix in fume hood!!)

10% formalin in PBS (pH 7.4)

Mix the following solutions to prepare the fixative:

<table>
<thead>
<tr>
<th>Solution</th>
<th>Amount</th>
<th>Part</th>
</tr>
</thead>
<tbody>
<tr>
<td>formalin (pH 35-37 % formaldehyde)</td>
<td>1 part</td>
<td></td>
</tr>
<tr>
<td>d.d. H2O</td>
<td>8 parts</td>
<td></td>
</tr>
<tr>
<td>10 x PBS stock</td>
<td>1 part</td>
<td></td>
</tr>
</tbody>
</table>

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<thead>
<tr>
<th>Solution</th>
<th>Amount</th>
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</tr>
</thead>
<tbody>
<tr>
<td>formalin</td>
<td>10 ml</td>
<td>5 ml</td>
</tr>
<tr>
<td>d.d. H2O</td>
<td>60 ml</td>
<td>40 ml</td>
</tr>
<tr>
<td>10 x PBS stock</td>
<td>10 ml</td>
<td>5 ml</td>
</tr>
</tbody>
</table>

Total volume: 100 ml