# Recipes for Groves Lab Stocks and Solutions

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**4% Paraformaldehyde**

!! Use a mask and gloves to weigh out paraformaldehyde !!

1. Add 40g paraformaldehyde (JT Baker, cat# S898) to 1 liter of 1 x PBS in a 1 liter bottle. Add 1ml of 0.1M NaOH.

2. Place the bottle in a 70°C water bath, inverting the bottle a few times every 5 minutes until the paraformaldehyde is completely dissolved. If the paraformaldehyde not dissolving completely, a little more NaOH will help.

3. Let the solution cool to room temperature.

4. Adjust the pH of the solution to 7.2 with conc. HCl.

5. Dispense out in 10ml aliquots and freeze at -20°C.

N.B. 4% paraformaldehyde is different from the “Neutral Buffered 10% Formalin” solution that can be purchased from many suppliers. Formaldehyde is a gas, which is typically dissolved in water to a concentration of 37%, with 10% methanol added as a stabilizer. This is “formalin”. “10% formalin” contains just under 4% formaldehyde, but also contains 1% methanol.

4% paraformaldehyde works better for most applications is our lab.

For more details, see http://publish.uwo.ca/~jkiernan/formglut.htm
5 x MABT

58g Maleic Acid

43.5g NaCl

55g Tween-20

900ml water

Bring the pH to 7.5 by adding Tris base. About 100g will be required. Bring final volume to 1 liter.
10 x PBS

For a 7 liter carboy:

560g NaCl

14g KCl

100.8g Na$_2$HPO$_4$

16.8g KH$_2$PO$_4$

6 liters water.

Bring pH to 7.4 and adjust volume to 7 liters
**10 x TBE**

54g Tris Base (Not Tris-HCl)

27.5g Boric Acid

20ml 0.5M EDTA, pH 8.0

Make up to 1 liter and autoclave.

N.B. This recipe is actually for what used to be called “5x TBE” in older protocols, with the final solution being a 10-fold dilution to “0.5x TBE”. To make things less confusing, many people (including our lab) now refer to this stock as 10 x TBE.
**20 x SSC**

*For a 1 liter:*

175.3 g NaCl

88.2 g Sodium Citrate

750ml water

Stir to dissolve the salts, and then add 5M NaOH dropwise to bring the pH to 7.0. Make up to 1 liter with more water. Autoclave.
**50 x TAE**

**For a 7 liter carboy:**

1694g Tris Base (Not Tris-HCl)

400ml Glacial Acetic Acid

500ml 0.5M EDTA (pH8.0)

Water to 7 liters
Ampicillin

For a 100mg/ml stock:

4g Ampicillin sodium salt

Dissolve in 40g water.

Filter through a 0.22µm filter and store in 1ml aliquots in the dark at -20°C.
**Chloramphenicol**

Make a 15mg/ml solution of Chloramphenicol in 100% Ethanol and store 1ml aliquots in the dark at -20°C.
**Howard Ringer’s Solution**

<table>
<thead>
<tr>
<th>Solute</th>
<th>1 liter</th>
<th>5 liters</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>7.2g</td>
<td>36g</td>
</tr>
<tr>
<td>CaCl₂</td>
<td>0.17g</td>
<td>0.85g</td>
</tr>
<tr>
<td>[ or CaCl₂.2H₂O</td>
<td>0.225</td>
<td>1.125g</td>
</tr>
<tr>
<td>KCl</td>
<td>0.37g</td>
<td>1.85g</td>
</tr>
<tr>
<td>Water</td>
<td>to 1 liter</td>
<td>to 5 liters</td>
</tr>
</tbody>
</table>

**Bottles for Howard Ringer’s Solution**

It is very important to keep the 100ml bottles for Howard Ringer’s separate from the regular lab glassware. They should never be exposed to detergents of any kind.

To refill the bottles, rinse them in distilled water and dry at 60°C overnight. Autoclave the bottles and caps and then filter the Ringer’s solution into them in a culture hood using a 0.22µm vacuum filter.
**Kanamycin**

Make a 20mg/ml solution of Kanamycin in distilled water. Filter through a 0.22µm filter and store in 1ml aliquots in the dark at -20°C.
Proteinase K

Make up a 25mg/ml solution of Proteinase K (Sigma P-6556) in PBS. Dispense into 50µl aliquots and freeze at -20C.
Roche Blocking Reagent

N.B. This used to be sold by Boehringer Mannheim, so it is sometimes referred to as “Boehringer Blocking Reagent” or “BBR”

4.4 g NaCl

5.8 g Maleic Acid

450 ml water

Bring to pH 7.5 by adding 5M NaOH dropwise.

Bring to 500ml with water.

Add one bottle (50g) of Blocking Reagent.

Stir constantly on a hot plate to dissolve the powder. You will need to bring the solution close to boiling to dissolve the powder. Be very careful not to let the solution boil over, as it really messes up the hot plate! It’s best to use a 1 or 2 liter beaker for this.
**SOC for bacterial transformation**

20g Bacto-tryptone

5g Bacto-yeast extract

0.5g NaCl

950ml water.

Shake until all the solutes have dissolved.

Add 10ml of 250mM KCl (1.86g KCl in 100ml water)

Adjust the pH to 7.0 with 5M NaOH.

Adjust the volume to 1 liter with water and autoclave

When the solution is cool, add 20ml of sterile 1M glucose solution (see below) in a cell culture hood and dispense out in 50ml aliquots.

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**1M Glucose (Sterile)**

18g glucose

90ml water.

Dissolve the glucose and adjust the volume to 100ml with water.
Sterilize the solution by filtration through a 0.22µm filter
**Whole Mount in situ hybridization buffer**

**For 1 liter of buffer:**

- **Formamide** (Good quality – from Ambion or Applied Biosystems) 500ml
- **20 x SSC, pH 4.5** 75ml
- **Yeast tRNA stock** 2.5ml
- **Heparin Stock** 2ml
- **Tween 20** 2ml
- **CHAPS (Sigma C-3023)** 5g
- **0.2M EDTA** 25ml
- **DEPC-Water** 393.5

Mix in a sterile plastic roller culture bottle (we use 1L Corning bottles) and dispense out in 50ml aliquots. Store at -20°C.

**20 X SSC pH4.5**

Take 500ml lab stock of 20 x SSC and adjust pH to 4.5 with citric acid. Add 0.5ml DEPC and incubate overnight at 37°C. Autoclave.

**Yeast tRNA stock**

Make a 20mg/ml solution of yeast tRNA (Sigma R-8759) in 50% formamide (made with DEPC-treated water). Store at -20°C.

**Heparin stock**

Make a 50mg/ml solution of heparin (Sigma H-3393) in 50% formamide (made with DEPC-treated water). Store at -20°C.

**0.2M EDTA**

Dissolve 37.22g EDTA (Disodium salt, dihydrate) in 450ml water. You will need to add 5M NaOH dropwise to get it to dissolve and to bring it to pH8.0. Bring solution to 500ml with water. Add 0.5ml DEPC and incubate overnight at 37°C. Autoclave.
### Section in situ hybridization buffer

**For 1 liter of buffer:**

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formamide (Good quality – from Ambion or Applied Biosystems)</td>
<td>500ml</td>
</tr>
<tr>
<td>20 x SSC (ordinary lab stock, DEPC-treated and autoclaved)</td>
<td>250ml</td>
</tr>
<tr>
<td>Yeast tRNA stock</td>
<td>2.5ml</td>
</tr>
<tr>
<td>Heparin Stock</td>
<td>2ml</td>
</tr>
<tr>
<td>Tween 20</td>
<td>1ml</td>
</tr>
<tr>
<td>100x Denhardt’s solution</td>
<td>10ml</td>
</tr>
<tr>
<td>CHAPS (Sigma C-3023)</td>
<td>1g</td>
</tr>
<tr>
<td>0.2M EDTA</td>
<td>25ml</td>
</tr>
<tr>
<td>DEPC-Water</td>
<td>210ml</td>
</tr>
</tbody>
</table>

Mix in a sterile plastic roller culture bottle (we use 1L Corning bottles) and dispense out in 50ml aliquots. Store at -20°C.

### 100x Denhardt's Solution

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>2% BSA (ICN 810661)</td>
<td></td>
</tr>
<tr>
<td>2% Polyvinylpyrrolidone (PVP-40)</td>
<td></td>
</tr>
<tr>
<td>2% Ficoll 400</td>
<td></td>
</tr>
</tbody>
</table>

Make a slurry in DEPC-water by rocking on a platform for several hours and dilute.
**X-Gal solution**

Make a 25 mg/ml solution of X-Gal in Dimethyl Formamide (DMF; use gloves!).

Store 1 ml aliquots at -20°C.

N.B. Some people use DMSO instead of DMF. We prefer DMF because the yellowing of the X-Gal solution occurs much more slowly in DMF.