A1. Buffers and reagents for DNA manipulation

**CTAB extraction solution**
2% (w/v) CTAB
100 mM Tris.HCl, pH 8.0
20 mM EDTA, pH 8.0
1.4 M NaCl

**CTAB precipitation solution**
1% (w/v) CTAB
50 mM Tris.HCl, pH 8.0
10 mM EDTA, pH 8.0

**Extraction buffer**
100 mM Tris.HCl, pH 8.0
100 mM EDTA, pH 8.0
250 mM NaCl
100 µg/ml proteinase K (add fresh before use)

**High-salt TE buffer**
10 mM Tris.HCl, pH 8.0
0.1 mM EDTA, pH 8.0
1 M NaCl

**TAE (Tris/acetate/EDTA) electrophoresis buffer**

50× stock solution: Working solution, pH 8.5:
242 g Tris base 40 mM Tris.acetate
57.1 ml glacial acetic acid 2 mM Na₂EDTA.2H₂O
37.2 g Na₂EDTA.2H₂O H₂O to 1 liter

**TBE (Tris/borate/EDTA) electrophoresis buffer** (10× stock solution, 1 liter)
108 g Tris base (890 mM)
55 g boric acid (890 mM)
40 ml 0.5 M EDTA, pH 8.0

**EDTA (0.5 M, pH 8.0):**
Add 186.1 g of disodium EDTA•2H₂O to 800 ml of H₂O. Stir vigorously on a magnetic stirrer. Adjust the pH to 8.0 with NaOH. Sterilize by autoclaving. The disodium salt of EDTA will not go into solution until the pH of the solution is adjusted to approx. 8.0 by the addition of NaOH.

**TE (Tris/EDTA) buffer**
10 mM Tris.HCl, pH 7.4, 7.5, or 8.0
1 mM EDTA, pH 8.0
**Sodium acetate, 3 M**
Dissolve 408 g sodium acetate·3H₂O in 800 ml H₂O. Add H₂O to 1 liter
Adjust pH to 4.8 or 5.2 (as desired) with 3 M acetic acid

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**A2. Buffers and reagents for RNA isolation and gel electrophoresis**

**Diethylpyrocarbonate (DEPC) treatment of solutions**
Add 0.2 ml DEPC to 100 ml of the solution to be treated. Shake vigorously to get the DEPC into solution. The solutions were autoclaved to inactivate the residual DEPC. Sodium acetate, water, and LiCl solutions should be treated with DEPC to inactivate RNase.

**Grinding buffer**
0.18 M Tris
0.09 M LiCl
4.5 mM EDTA
1% sodium dodecyl sulfate (SDS)
pH to 8.2 with HCl

**TLE solution**
0.2 M Tris
0.1 M LiCl
5 mM EDTA
pH to 8.2 with HCl

**Phenol**
Equilibrate freshly liquefied phenol (250 ml for a 15-g prep) with TLE solution on the day of preparation. First, extract with an equal volume of TLE solution plus 0.5 ml of 15 M NaOH (this should bring the pH close to 8.0), then extract two more times with TLE solution.

**RNA loading buffer**
80% (v/v) formamide 1 mM EDTA, pH 8.0
0.1% bromphenol blue
0.1% Xylene Cyanol

**MOPS buffer**
0.2 M MOPS [3-(N-morpholino)propanesulfonic acid], pH 7.0
0.5 M sodium acetate
0.01 M EDTA
A3. For bacterial transformation and plasmid preparation

Media and Solutions

LB (Luria-Bertani) medium (1 liter)
10 g tryptone
5 g yeast extract
5 g NaCl
Adjust pH to 7.5 with NaOH and autoclave.
For solid medium, please see Media Containing Agar or Agarose (1.5%).

SOC medium
2.0 g tryptone
0.5 g yeast extract
1ml 1M NaCl
0.25ml 1M KCl
1ml Mg$^{2+}$ stock (1M MgCl$_2$ • 6H$_2$O, 1M MgSO$_4$•7H2O) filter-sterilized
1ml 2 M glucose, filter-sterilized
Add tryptone, yeast extract, NaCl and KCl to 97ml distilled water. Stir to dissolve.
Autoclave and cool to room temperature. Add 2M Mg2+ stock and 2M glucose stock, each to a final concentration 20mM. Adjust to pH 7.0. Filter the complete medium through a 0.2 µm filter unit.

Ampicillin Stock Solution:
Dissolve at 100mg/ml in water, filter sterilize, store in aliquots at –20 °C. Final concentration of 100 µg/ml

IPTG stock solution (0.1M):
Weigh 1.2 g IPTG and dissolve it in deionized distilled water to 50ml final volume. Filter-sterilize and store at 4 °C.

X-Gal stock solution (20 mg/mL):
100mg of X-gal dissolved at 20mg/ml in N,N′-dimethyl-formamide. Cover with aluminum foil and store at –20°C. Final concentration of 80µg/mL X-Gal for blue-white selection.

Glycerol [10% (v/v)] solution: Dilute 1 volume of molecular-biology grade glycerol in 9 volumes of sterile pure H$_2$O. Sterilize the solution by passing it through a pre-rinsed 0.22-µm filter. Store in 200-ml aliquots at 4 °C.

Solution I
50 mM glucose
25 mM Tris.HCl (pH 8.0)
10 mM EDTA (pH 8.0)
Prepare Solution I from standard stocks in batches of approx. 100 ml, autoclave for 15 minutes at 15 psi (1.05 kg/cm²) on liquid cycle, and store at 4 °C.

**Alkaline Lysis Solution II**
0.2 N NaOH (freshly diluted from a 10 N stock)
1% (w/v) SDS
Prepare Solution II fresh and use at room temperature.

**Potassium Acetate neutralization solution III**
5 M potassium acetate, 60 mL
glacial acetic acid, 11.5 mL H₂O, 28.5 mL
The resulting solution is 3 M with respect to potassium and 5 M with respect to acetate.
Store the buffer at room temperature.

**A4. Reagents and buffers for protein isolation, SDS-PAGE and immune blotting**

**SDS electrophoresis buffer, 5x**
15.1 g Tris base
72.0 g glycine
5.0 g SDS
H₂O to 1000 ml

**SDS-PAGE sample buffer**
15% (v/v) glycerol
0.125 M Tris.HCl, pH 6.8
5 mM Na₂EDTA
2% (w/v) SDS
0.1% (w/v) bromphenol blue
1% (v/v) 2-mercaptoethanol (2-ME; add immediately before use)

**Alkaline phosphate substrate buffer**
100 mM Tris.Cl, pH 9.5 100 mM NaCl 5 mM MgCl₂

**BCIP/NBT visualization solution**
Mix 33 µl NBT stock (100 mg NBT in 2 ml at 70% DMF, stored <1 year at 4°C) and 5 ml alkaline phosphate substrate buffer (see recipe). Add 17 µl BCIP stock (100 mg BCIP in 2 ml of 100% DMF, <1 year at 4°C) and mix.

**Blocking buffer**
*For nitrocellulose and PVDF*: 0.1% (v/v) Tween 20 in TBS (TTBS).
*For neutral and positively charged nylon*: Tris-buffered saline (TBS) containing 10% (w/v) nonfat dry milk.
**Ponceau S solution**
Dissolve 0.5 g Ponceau S in 1 ml glacial acetic acid. Bring to 100 ml with water. Prepare just before use.

**Transfer buffer**
Add 18.2 g Tris base and 86.5 g glycine to 4 liters of water. Add 1200 ml methanol and bring to 6 liters with water. The pH of the solution is ~8.3 to 8.4. For use with PVDF filters, decrease methanol concentration to 15%; for nylon filters, omit methanol altogether.

**Buffers and Reagents for ELISA:**
Bicarbonate/carbonate coating buffer (100 mM) for dilution antigens and subsequent immobilization to the wells in microtitre plate: 3.03 g Na2CO3, 6.0 g NaHCO3 1000 ml distilled water pH 9.6.
Blocking solution: blocking agents are 1% BSA, serum, non-fat dry milk, gelatin in PBS.
Wash solution: Usually PBS or Tris-buffered saline (pH 7.4) with detergent such as 0.05% (v/v) Tween20 (TBST).
Antibody dilution buffer: Primary and secondary antibody should be diluted in 1x blocking solution to reduce Non specific binding.

### A5. Recipes of common buffers

**PBS (phosphate-buffered saline)**

10× *stock solution, 1 liter:*

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>80 g/liter</td>
</tr>
<tr>
<td>KCl</td>
<td>2 g/liter</td>
</tr>
<tr>
<td>Na2HPO4.7H2O</td>
<td>11.5 g/liter</td>
</tr>
<tr>
<td>KH2PO4</td>
<td>2 g/liter</td>
</tr>
</tbody>
</table>

**Working solution, pH 7.3:**

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>137 mM</td>
</tr>
<tr>
<td>KCl</td>
<td>2.7 mM</td>
</tr>
</tbody>
</table>

**Tris-buffered saline (TBS)**

100 mM Tris.Cl, pH 7.5

0.9% (150 mM) NaCl

Store up to several months at 4°C

**Sodium acetate buffer, 0.1 M**

*Solution A:* 11.55 ml glacial acetic acid/liter (0.2 M).

*Solution B:* 27.2 g sodium acetate (NaC2H3O2·3H2O)/liter (0.2 M).

Referring to Table A.2.2 for desired pH, mix the indicated volumes of solutions A and B, then dilute with H2O to 100 ml.

**Sodium phosphate buffer, 0.1 M**

*Solution A:* 27.6 g NaH2PO4·H2O per liter (0.2 M).

*Solution B:* 53.65 g Na2HPO4·7H2O per liter (0.2 M).
Referring to Table A.2.3 for desired pH, mix the indicated volumes of solutions A and B, then dilute with \( \text{H}_2\text{O} \) to 200 ml.

**NaCl, 5 M**
292 g NaCl in 1 liter \( \text{H}_2\text{O} \)

**NaOH, 10 M**
Dissolve 400 g NaOH in 450 ml \( \text{H}_2\text{O} \). Add \( \text{H}_2\text{O} \) to 1 liter

**Dithiothreitol (DTT), 1 M**
Dissolve 15.45 g DTT in 100 ml \( \text{H}_2\text{O} \). Store at −20 °C

**EDTA (ethylenediamine tetraacetic acid), 0.5 M (pH 8.0)**
Dissolve 186.1 g \( \text{Na}_2\text{EDTA} \cdot 2\text{H}_2\text{O} \) in 700 ml \( \text{H}_2\text{O} \)
Adjust pH to 8.0 with 10 M NaOH
Add \( \text{H}_2\text{O} \) to 1 liter

**Ethidium bromide, 10 mg/ml**
Dissolve 0.2 g ethidium bromide in 20 ml \( \text{H}_2\text{O} \). Mix well and store at 4 °C in dark

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### A6. Buffers and reagents for immunoaffinity chromatography

**Column storage solution**
Prepare in TSA solution either 1 mM EDTA/20 µg/ml gentamicin or 0.01% thimerosal.

**Detergent stock solutions**
Prepare 10% Triton X-100 or 5% sodium deoxycholate in water. Sterilize either solution by Millipore filtration. Triton X-100 solution should be stored in the dark to prevent photooxidation. **NP-40 can be used in place of Triton X-100.**

**Glycine buffer**
50 mM glycine-HCl, pH 2.5
0.1% Triton X-100
0.15 M NaCl

**Lysis buffer**
**TSA solution containing:** 2% Triton X-100, 5 mM iodoacetamide, Aprotinin (0.2 trypsin inhibitor U/ml) 1 mM phenylmethylsulfonyl fluoride (add fresh from 100 mM stock solution prepared in absolute ethanol)

**Sodium phosphate buffer, pH 6.3**
50 mM sodium phosphate, pH 6.3
0.1% Triton X-100
0.5 M NaCl
Triethanolamine solution
50 mM triethanolamine, pH ∼11.5
0.1% Triton X-100 0.15 M NaCl

Tris/saline/azide (TSA) solution
0.01 M Tris-Cl, pH 8.0 (at 4 °C)
0.14 M NaCl
0.025% NaN₃

Tris/Triton/NaCl buffer, pH 8.0 and 9.0
50 mM Tris-Cl, pH 8.0 or pH 9.0
0.1% Triton X-100
0.5 M NaCl

Wash buffer
0.01 M Tris-Cl, pH 8.0 (at 4 °C)
0.14 M NaCl
0.025% NaN₃
0.5% Triton X-100
0.5% sodium deoxycholate

A7. Hoagland’s Solution

<table>
<thead>
<tr>
<th>Component</th>
<th>Stock Solution</th>
<th>mL Stock solution/1L</th>
</tr>
</thead>
<tbody>
<tr>
<td>2M KNO₃</td>
<td>202g/L</td>
<td>2.5</td>
</tr>
<tr>
<td>2M Ca(NO₃)₂ x 4H₂O</td>
<td>236g/0.5L</td>
<td>2.5</td>
</tr>
<tr>
<td>Iron</td>
<td>15g/L</td>
<td>1.5</td>
</tr>
<tr>
<td>2M MgSO₄ x 7H₂O</td>
<td>493g/L</td>
<td>1</td>
</tr>
<tr>
<td>1M NH₄NO₃</td>
<td>80g/L</td>
<td>1</td>
</tr>
<tr>
<td>Minors:</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>H₃BO₃</td>
<td>2.86g/L</td>
<td></td>
</tr>
<tr>
<td>MnCl₂ x 4H₂O</td>
<td>1.81g/L</td>
<td></td>
</tr>
<tr>
<td>ZnSO₄ x 7H₂O</td>
<td>0.22g/L</td>
<td></td>
</tr>
<tr>
<td>CuSO₄</td>
<td>0.051g/L</td>
<td></td>
</tr>
<tr>
<td>H₂MoO₄ x H₂O or Na₂MoO₄ x 2H₂O</td>
<td>0.09g/L</td>
<td></td>
</tr>
<tr>
<td>1M KH₂PO₄ (pH to 6.0 with 3M KOH)</td>
<td>136g/L</td>
<td>0.5</td>
</tr>
</tbody>
</table>

1) Make up stock solutions and store in separate bottles with appropriate label.
2) Add each component to 800mL deionized water then fill to 1L.
3) After the solution is mixed, it is ready to water plants.
Table A1. Protein standard curve (Bradford colorimetric assay)

\[ y = 0.036x \]
\[ R^2 = 0.992 \]