PROGRAMMED CELL DEATH IN PROKARYOTES: AN INSIGHT INTO THE TOXIN-ANTITOXIN MODULE OF Bacillus anthracis

APPENDIX
PREPARATION OF BACTERIAL CULTURE MEDIA

**LB medium (Luria Broth)**

Dissolve 20 gm of LB powder (Hi Media) in MQ. Sterilize the media by autoclaving for 20 min at 15 lb/sq.in.

**LB Agar**

Dissolve 35 gm of LB agar powder (Hi Media) in double distilled water. Sterilize the media by autoclaving for 20 min at 15 lb/sq.in. Allow LB agar to cool and pour in 90 mm disposable petri plates (Tarsons) along with appropriate antibiotics and allow it to solidify.

**ANTIBIOTICS SOLUTIONS**

**Ampicillin**

Prepare 100 mg/ml stock in a/c MQ and store by freezing at -20 °C.

**Kanamycin**

Prepare 50 mg/ml stock solution in a/c MQ and store by freezing at -20 °C.

**SOLUTIONS FOR PLASMID ISOLATION AND PURIFICATION**

**Solution I**

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Component</th>
</tr>
</thead>
<tbody>
<tr>
<td>50 mM</td>
<td>Glucose</td>
</tr>
<tr>
<td>25 mM</td>
<td>Tris-Cl (pH 8.0)</td>
</tr>
<tr>
<td>10 mM</td>
<td>EDTA</td>
</tr>
</tbody>
</table>

Prepare Solution I in batches of 100ml, a/c for 20 min at 15 lb/sq.in. and store at 4 °C.

**Solution II**

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Component</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.2 N</td>
<td>NaOH (freshly diluted from 10 N stock).</td>
</tr>
<tr>
<td>1 %</td>
<td>SDS</td>
</tr>
</tbody>
</table>

**Solution III**

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Component</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 M</td>
<td>Potassium acetate</td>
</tr>
<tr>
<td>60 ml</td>
<td>Glacial Acetic acid</td>
</tr>
<tr>
<td>11.5 ml</td>
<td>MQ</td>
</tr>
<tr>
<td>28.5 ml</td>
<td></td>
</tr>
</tbody>
</table>

The resulting solution is 3 M with respect to potassium and 5 M with respect to acetate. a/c at 15 lb/sq.in. for 20 min. Store at 4 °C.
STOCK SOLUTIONS OF COMMONLY USED REAGENTS

1 M Tris.
Dissolve 121.1 gm of Tris base in 800 ml of MQ and adjust the desired pH (6.8, 7.4, 8.0, 8.8) with concentrated HCl. Make up the volume to 1 liter and autoclave.

0.5 M EDTA.
Add 186.1 gm of disodium EDTA.2 H2O in 800 ml of MQ. Stir vigorously on a stirrer, adjust the pH to 8.0 with NaOH (about 20 gm of NaOH pellets), make up the volume to 1 liter and autoclave.

3 M sodium acetate
Dissolve 204.5 gm of C2H3O2Na.3H2O in 400 ml of MQ. Adjust the pH to 5.3 with glacial acetic acid. Make up the volume to 500 ml and autoclave.

10 % SDS
Dissolve 10 gm of electrophoresis grade SDS in 70 ml of MQ heat at 60 °C to dissolve and make up the volume to 100 ml.

Ethidium Bromide (10 mg/ml)
Dissolve 10 mg of ethidium bromide in 1 ml MQ. Store in a dark bottle.

30 % Acrylamide Stock
Dissolve 29.2 gm of acrylamide and 0.8 gm of bis-acrylamide in 50 ml of MQ. Make up the volume to 100 ml, filter the solution through Whatman no. 1 paper and store in a dark bottle.

Calcium Chloride (0.1 M)
Dissolve 147.0 gm of CaCl2.2H2O in 100 ml of MQ and sterilize by autoclaving.

IPTG (1 M)
Dissolve 238 mg of IPTG in 1 ml of MQ. Filter Sterilize and store at -20 °C.
Sodium Phosphate (1 M)

**Monobasic**

Dissolve 138 gm of NaH₂PO₄·H₂O in 800 ml of MQ and make up the volume to 1 liter.

**Dibasic**

Dissolve 268 gm of Na₂HPO₄·7H₂O in 700 ml of MQ and make up the volume to 1 liter.

**Ammonium persulfate (10 %)**

To 1 gm of ammonium persulfate add 10 ml of MQ and store at 4 °C.

**100 mM Phenyl methyl-sulfonyl fluoride**

Dissolve 17.4 mg of PMSF in 1 ml of isopropanol. Store at -20 °C.

**BUFFERS**

**50 x TAE buffer (Tris-acetate, EDTA)**

Dissolve 242 gm of Tris base in 700 ml of MQ and add 57.1 ml of glacial acetic acid and 100 ml of 0.5 EDTA pH 8.0. Make up the final volume to 1 liter.

**10 x TBE buffers (Tris borate, EDTA)**

Dissolve 8 gm of Tris base, 55 gm of boric acid and 9.3 gm Na₂EDTA·H₂O in 700 ml MQ and make up the final volume to 1 liter.

**Phosphate Buffer Saline (PBS)**

Dissolve 8 gm of NaCl, 2 gm of KCl, 1.44 gm of Na₂HPO₄ and 0.2 gm of KH₂PO₄ in 800 ml of MQ. Adjust the pH to 7.4 with HCl. Make up the final volume to 1 liter and sterilize by autoclaving at 15 lb/sq.in for 20 min and store at room temperature.

**SDS-PAGE electrophoresis buffer**

Dissolve 3 gm of Tris base, 14.4 gm of glycine and 1 gm of SDS in 1 liter MQ.

**Electrode transfer buffer**

Dissolve 5.8 gm of Tris base, 2.9 gm of glycine and 0.33 gm of SDS in 0.5 liter of MQ. Add 200 ml of ethanol and make up the final volume to 1 liter.
2 × SDS-PAGE sample buffer

The composition of sample buffer is as follows

- 100 mM Tris-Cl (pH 6.8)
- 200 mM DTT
- 4% SDS
- 0.2% Bromophenol blue
- 20% Glycerol
- 10% β-mercaptoethanol

6 × DNA loading dye

Dissolve 0.2 gm bromophenol blue, 0.2 gm xylene cyanol and 30 ml of glycerol and make up the volume to 100 by a/c MQ.

SDS-PAGE buffers

Composition of resolving gel (12 %) 10 ml

- 4.0 ml 30% acrylamide solution
- 2.5 ml 1.5 M Tris-Cl pH 8.8
- 3.3 ml MQ
- 100 μl 10% SDS
- 100 μl 10% APS
- 10 μl TEMED

Composition of resolving gel (15 %) 10 ml

- 5.0 ml 30% acrylamide solution
- 2.5 ml 1.5 M Tris-Cl pH 8.8
- 2.3 ml MQ
- 100 μl 10% SDS
- 100 μl 10% APS
- 10 μl TEMED

Composition of stacking gel (5 %) (5.0 ml)

- 0.83 ml 30% acrylamide solution
- 0.68 ml 1.0 M Tris.Cl pH 6.8
- 3.4 ml MQ
- 50 μl 10% SDS
- 50 μl 10% APS
- 5 μl TEMED
Staining solution

Dissolve 1 gm of coomassie blue in 450 ml of methanol. Add 100 ml of glacial acetic acid and make up the volume to 1 liter by double distilled water. Filter through Whatman no. 1 and store at room temperature.

Destaining solution


Silver staining reagents

Fixative: 40 ml Methanol, 10 ml Glacial Acetic Acid, 50 ml MQ
AgNO₃: weigh 0.2 g of AgNO₃ in dark and 75 µl of formaldehyde in 100 ml
Hypo: 50 ml 0.02 % sodium thiosulfate
Developer: 50 ml 2 % Na₂CO₃ and 50 µl formaldehyde (add during the procedure), store at 4 °C till it is used.
Stop solution: Sodium citrate 2.1 g for 100 ml